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Research Strategies for the Application of the
Techniques of Computational Biological
Chemistry to Environmental Problems

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RESEARCH STRATEGIES FOR THE APPLICATION OF THE TECHNIQUES OF COMPUTATIONAL
BIOLOGICAL CHEMISTRY TO ENVIRONMENTAL PROBLEMS

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Recommendations from a workshop sponsored by the
Genetic Toxicology Division
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Organized and edited by

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EXECUTIVE SUMMARY

On October 25 and 26, 1984, the U.S. EPA sponsored a workshop to consider the potential applications of the techniques of computational biological chemistry to problems in environmental health. Eleven extramural scientists from the various related disciplines and a similar number of Agency personnel participated. Briefing materials describing some specific Agency problems in this area were sent to the extramural scientists before the meeting. The workshop's goal was the development of a research strategy for these potential applications.

At this workshop, four classes of problems were identified and the recommendations of the panel for each class were drafted separately. The four classes were as follows: 1) methodology for handling diverse structures, 2) methodology for handling data sets with substantial structural similarities, 3) theoretical structure-activity studies using causally related molecular parameters, and 4) application possibilities for artificial intelligence decision aids in the existing Premanufacture Notification (PMN) review process. The final document was a product of the entire panel. All members agreed on the need for experimental toxicity data sets designed for structure-activity relationship studies.

Several currently available methods assist in the evaluation of chemical toxicity for sets of chemicals with diverse structures. These methods are based on the establishment of correlations between biological activity and chemical features. They differ in their operational algorithms and in the nature and method of choosing relevant chemical features. Data bases containing information about hundreds of tested chemicals are necessary to establish structure-activity correlations for diverse chemical structures. It is essential that the biological activity is consistently measured in controlled experiments and that a wide range of activity is represented in the data set. The available methods for establishing correlations are regression analysis, pattern recognition, cluster analysis, discriminant analysis, and automated evaluation based on substructure. It is often difficult to establish causal relationships between structure and activity based on the correlations generated by these methods, but this should remain the ultimate goal.

When sets of chemicals have structural similarities, methods that take advantage of this similarity may be used. They require a smaller data base, on 20-200 chemicals, and provide a quantitative basis for comparison of tested and untested chemicals. The methods discussed were molecular superposition, correlational analysis, ADAPT, CASE, and graph-theoretical approaches. These methods use molecular shape, physicochemical properties, and topological, electronic, and substructural features of molecular structure as their independent elements. They identify the most important elements or the relationship between elements from the measured activities of previously tested chemicals by using various statistical, pattern recognition, correlative, and ad hoc techniques. These results may then be used to predict the activity of chemicals structurally similar to the previously tested chemicals. The degree of similarity to previously tested chemicals can be determined by multivariate statistical techniques. The panel suggested that studies of methodologies that develop models and the development of suitable experimental data sets are needed.

The development and application of information about the mechanisms of specific types of toxicity in specific chemical classes is an important element in the prediction of toxicity. The techniques of theoretical chemistry can be useful in elucidating these mechanisms, in the selection of molecular descriptors relevant to a particular risk, and in the calculation of selected molecular descriptors potentially useful in screening large numbers of molecules. These techniques can be used to characterize individual molecules, complex formation between a molecule and its site of action, and chemical reactions by the calculation of structural, electronic, and chemical reactivity properties.

Causal descriptors may also be obtained from experimental methods. The set of causal descriptors can be used to predict the activity of untested chemicals, and these predictions can be compared with experimental findings. This additional information can be used to strengthen the causal hypothesis of action or as an indication of the value of the theoretical model for screening large numbers of similar chemicals.

Artificial intelligence methods, such as expert systems, work best when applied to well-established reasoning problems that are repetitious. The PMN review is an example of such a problem. The process has been decomposed into nine steps, and the relevant artificial intelligence decision-making aids and the difficulty in their application has been reviewed for each step. Capturing the expertise of toxicologists on the PMN panel and elsewhere in an expert system could be very useful. A knowledge engineer could observe the panel meetings to develop rules and an expert system could be built over time operating in parallel with the panel, so that results could be evaluated and improved. Computer analysis for possible metabolic products exists. Expert systems approaches for searching data bases and assessing the similarity between chemicals were discussed.

All panel members agreed on the need for systematic experimental studies and development of data bases for the relevant forms of activity. The computational theoretical techniques discussed must interact with experimental information. Increased knowledge of the underlying cause of the relevant activities will increase the accuracy and applicability of these techniques.

INTRODUCTION

Reliable methods for assessing the possible health effects of chemicals when they are not available for bioassay or in situations for which relevant bioassays are incomplete would be of great value to the U.S. Environmental Protection Agency. These methods could be used in developing regulatory, testing, and research strategies. Methods of this type would be of particular importance for the agency's premanufacture notification program.

Computational techniques have been used for many years to predict chemical properties from chemical structure. These techniques have been used to provide insight into the reasons for the biological activity of some chemicals and to design biologically active molecules. The recent development of computational methods and equipment has led to considerable use of these techniques in the pharmaceutical industry. As the capability of dealing with large molecules and our understanding of biological/environmental processes increases, the utility of these techniques for environmental problems grows.

A principal application of these techniques to problems in environmental health is the prediction of chemical toxicity from chemical structure. This application would be particularly useful for the evaluation of new chemicals accompanied by few, if any, test data on their health effects when they are submitted to the agency. This application is different from the use of these techniques to design biologically active chemicals (the goal in industry) in two significant ways. First, the prediction of health effects is not a single end point, but depends on multiple interaction paths that are often not well understood. Second, there are few experimental data available or easily obtainable, not only data relative to health and environmental effects, but also relative to all but the simplest physical properties, on new chemicals. Further, these chemicals are often not the simple extension of a family of similar molecules, as is often the case in pharmaceutical applications, for which a great deal of data exist for other members of the family.

A research effort to develop these computational techniques for problems in environmental health is interdisciplinary in nature and is in its formative stage, particularly as it relates to adverse health effects. For these reasons, it was deemed valuable to hold a workshop to develop a strategy for the application of the techniques of computational biological chemistry to problems of environmental health.

This workshop, held on October 25 and 26, 1984, brought together eleven outstanding scientists from outside the agency and agency regulatory and scientific personnel. The extramural personnel were selected to encompass the various disciplines in computational biological chemistry, not because of their previous experience with environmental problems. They all left the workshop with a better appreciation of the specific problems of the agency and general environmental problems. Before the workshop, the extramural participants received a series of briefing documents on agency problems prepared by agency personnel involved with those problems. These documents are part of this report and were used as background material. The stated goal of the workshop was not to offer specific solutions to any of these problems, but to develop a strategy to assess, develop, and apply the techniques of computational biological chemistry to problems in environmental health. However, some of the questions engendered by the briefing material are considered specifically in this report.

The first day of the workshop was spent considering the briefing material and discussing agency problems in general. At the end of the first day, the group divided into four subgroups based on the type of data potentially available and the type of techniques to be used. Each of these groups

produced a section of the report (sections 1-4). There was general agreement on the need for systematically obtained experimental data sets for the establishment and validation of the computational techniques.

The workshop closed with a plenary session in which the contribution of each subgroup was discussed by all the workshop participants. The report of the extramural panel follows.

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RECOMMENDATIONS OF THE PANEL

1. METHODOLOGY FOR HANDLING DIVERSE STRUCTURES

Introduction

In the last decade, several methods have emerged that have the potential for assisting in the evaluation of the possible toxicity of chemicals with diverse molecular structure. Most of these methods rely on possible correlations between biological activity and the presence of substructural features in the molecules of the data base. The methods differ in their operational algorithms and in the nature of and means of choosing descriptors.

Operational Algorithms--

These include pattern recognition techniques, stepwise regression analysis, cluster analyses, and discriminant analysis.

Nature of the Descriptors--

One major aspect of structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) studies is the selection of appropriate properties to be used as descriptors. The success of a QSAR study may depend on the outcome of an intelligent and properly discriminant analysis of the factors that may be "determinant" in the observed activities. The problem of choosing appropriate descriptors is particularly difficult to solve in the more heuristic approaches because of the need to find relevant descriptors of general utility for which values may be assigned for all chemicals under study.

The use of substructural units as descriptors makes considerable sense to chemists who are used to relating chemical properties to functionalities consisting of one to four atoms, e.g., COOH and NH₂. However, it is evident that biological functionality may entail larger fragments than the two or three atoms usually found in chemical functionalities.

Interesting information can often be gathered from the use of mathematical constructs derived from the presence of small clusters of atoms and bonds. However, direct mechanistic implications cannot easily be visualized from these descriptors. The use of larger substructural features as descriptors provides easily recognizable patterns for matching structure with biological functionality and may provide information about the mechanisms of action.

The establishment of causal relationships between structure and activity, while extremely difficult to prove, should remain the ultimate goal of these methodologies.

Open- vs. Closed-Ended Descriptors--

Most methodologies use a preselected set of descriptors, believed to be capable of describing biological activity in each data set. The presence of important chemical entities such as certain functional groups (e.g., N-NO) and their number (e.g., five C-Cl bonds) are examples of closed-ended descriptors. Some methods (Hodes and CASE methods) use an open-ended approach, which lets the computer decide the most appropriate descriptors from among all possible structural descriptors for the data set under consideration.

Requisite Size of the Data Set--

To establish structure-activity correlation, a "representative" data set is required. How small a data set can be representative? In development of LD₅₀ models, 400 to 500 compounds gave a significant model; there were some changes when 1000 compounds were used. Use of 1500 and 2000 compounds for development caused almost no changes in the model. Some useful models have been developed with as few as 200 chemicals, but these usually were not of widely diversified structure (e.g., nitrosamines).

Hence, if only a set of diverse structures is available for analysis, a minimum data set of several hundred compounds is necessary before modeling can succeed. Furthermore, it is highly desirable that the data set be evenly distributed between biologically "active" and "inactive" chemicals.

Need for the Data Set to have a Common Biological End Point--

It is essential that the biological end point be consistently measured in a controlled test and, preferably, carefully evaluated by an appropriate group of scientists. Where this is not possible, clustering techniques may help select a uniform data set by identifying individual outliers for removal.

A wide range of biological activity is desired for the chemicals in a data set because statistical methods such as regression analysis are based on the assumption of normal binomial distribution of the data. If only "active" and "inactive" rankings are available, approximately equal numbers of each are desirable.

Methods Available for Modeling

Regression Analysis Approach--

After a coherent group of diverse compounds has been identified, substructural features are assigned by algorithm (by using one of several available coding systems). The molecular weight and, if possible, other parameters such as the partition coefficient are added to the resulting structural parameters. Then, all parameters are included in the first-stage equation and, by backwards stepwise regression analysis, those parameters that contribute the least are dropped, one by one, until the removal of another parameter gives a significant loss of correlation. The resulting equation is statistically tested for robustness and is then used to predict the biological activity of a subset of compounds randomly withheld from the development data set. The results are compared with the experimental data to determine the utility of the model.

Pattern Recognition Approaches--

The initial pretreatment for ensuring uniform biological test data is followed by generation of several types of molecular structure descriptors:

- (1) Physical properties, e.g., partition coefficient, molecular volume, surface area
- (2) Electronic properties, e.g., charge distributions, molecular dipole moments calculated by simple, approximate methods
- (3) Topological features, e.g., substructural features defined above

These descriptors are then combined and comparisons are performed by pattern recognition methods. The data are used to generate discriminants by using multivariate statistical or pattern recognition methods. The results can be tested by randomly withholding subsets of compounds prior to development of discriminants and then comparing the ability of the discriminants to estimate the activities of the withheld sets. By randomly choosing subsets and repeating this process, one gains an understanding of the "stability" of the resulting discriminants.

The CASE Technique--

The Computer Automated Structure Evaluation (CASE) technique is used to automatically select the substructural units that are most appropriate for discrimination between active and inactive molecules within the data base under evaluation. These units can contain up to 12 heavy atoms (i.e., non-hydrogen atoms) plus the hydrogen atoms attached to them. Unlike the other methods, though, the CASE technique uses an open-ended approach to the selection of structural descriptors. In this respect it is an expert system capable of learning from the data.

The CASE program offers the possibility of testing compounds of unknown toxicity by comparing the subunits of the compound with those of the data base. In addition, for a data base in which there is quantitative, as well as qualitative, information about the biological activities of the compounds, this technique will produce a QSAR in which multivariate analysis is employed to assign numerical coefficients to the most significant activating and deactivating fragments. These coefficients may then be used to estimate the quantitative activity of known and unknown compounds.

The Hodes Method--

This approach was designed to use the thousands of test results obtained by screening diverse chemicals for antitumor activity as a guide in selecting additional candidates for testing. The chemical structures are entered by connection tables, and augmented connectivity fragments of several lengths and types are generated by algorithm. These fragments are weighted by their occurrence in active and inactive compounds, and these weights are used additively to predict activities of new chemicals in the biological test system.

2. METHODOLOGY FOR HANDLING DATA SETS WITH SUBSTANTIAL STRUCTURAL SIMILARITY

When sets of compounds have apparent structural similarities, one can take advantage of common structural features. This is in contrast to cases in which no apparent similarities exist and more general methods must be used. A typical analysis may include between 20 and 200 compounds, and the degree of similarity or diversity among the structures to be considered need not be rigidly limited. Methods described here can deliver the following:

- (1) A quantitative QSAR correlation between a moiety of interest and a biological activity
- (2) A quantitative bond-additive relationship
- (3) A quantitative parameter to "measure" the degree of similarity
- (4) A quantitative (objective) selection of analogues
- (5) Screening of a large collection of structures for prescribed (structure-activity) features

The methods differ in their specific characteristics; some are more general, others are suited for less heterogeneous classes (e.g., sensitive to alkyl substitution, but not sensitive to halogen substitution). They ought to be considered to some extent as complementary, rather than competitive. We will briefly review several methods in this section:

- (1) Molecular superposition
- (2) Correlation analysis
- (3) ADAPT
- (4) CASE
- (5) Graph-theoretical approaches

Molecular Superposition

Given a set of compounds with their associated activities, their electronic or steric commonalities can be assessed by superposition. The first step in this method is to construct three-dimensional molecular models (conformers) for each compound in the data set. The models can be generated by classical molecular mechanics methods or quantum mechanical methods. Then, given the set of molecular models, a superposition volume can be formed. This will express the volume occupied by all active compounds and the volume occupied by only inactive compounds (excluded volume). The hypothesis is that the activity of a new compound can be predicted by comparing its volume with the superposition volume. The method can be based on electrostatic charge distribution, rather than on molecular volume.

Correlation Analysis of Congeneric Sets

A large effort has been made in the past 20 years to correlate changes in chemical structure with changes in biological activity produced by modest sets of compounds (10 to 200) acting in well-defined systems, presumably by the same mechanism. At present, over 5000 such correlation equations have been developed for many chemicals acting on many types of biological systems, which range from isolated enzymes to human beings. This massive evidence has established that we

can formulate mathematical models for biological systems. The problem now is to improve the quality of this work and to place it on a more fundamental basis.

These correlations have largely been based on the combination (often, but not always, linear) of physicochemical parameters. The most widely used of these parameters have been obtained from model experimental systems. They have been developed to rationalize substituent effects on hydrophobic, electronic, and steric properties of a parent compound. Although quantum-chemically calculated parameters might have been used, so far relatively little effort has been made in this direction. Thus far, hydrophobic parameters, $\log P$, Π , or f values have been used. For electronic effects, the Hammett σ constant and its various forms and extensions have been most popular. Steric effects are, of course, the most difficult to deal with in mathematical terms. Because one usually does not know the shape of the receptor, there is as yet no general approach to steric effects. The commonly used steric parameters are Taft's E_s , molar refractivity (MR), and Verloop's sterimol constants.

Although correlation analysis seems to be thriving, there is need for support for the measurement of new $\log P$ values and the development of more satisfactory steric parameters.

ADAPT

Either quantitative models or discriminants can be developed by using calculated molecular structure descriptors for data sets with some structural diversity. The nature of the biological activity data available determines the type of mathematical model generated. The structural descriptors used fall into four classes: topological, geometric, electronic, and physicochemical. These descriptors are calculated, so structural diversity is no hindrance. Information about the mechanisms of action, metabolism, or other relevant processes may be taken into account as well. When each structure is represented by a common set of descriptors, pattern recognition or statistical analysis is used to seek relationships that statistically explain the biological activity. This approach is used to determine which structural descriptors are most useful in developing predictive relationships. Once a set of descriptors and an associated mathematical model or discriminant has been developed, it can be used to predict the biological activity of unknowns. The descriptors used in developing the model or discriminant can also be used to assess which of a set of models is most suitable for use in a particular area.

CASE

The CASE method is described in the previous section on diverse structures.

Graph-Theoretical Approaches

In graph-theoretical approaches, the graph represents molecular connectivity. Various schemes have been proposed in which structure is represented by a number (i.e., the so-called "topological index") or a sequence (such as path sequence). Topological indices have been directly used for correlations of chemical properties (and have been found useful for bond-additive properties). The connectivity index χ has been enlarged by supplementation with additional such indices, thus the use of this method approaches empirical curve-fitting techniques.

Path characterization represents a molecule by a sequence that is derived by counting special substructure elements (paths). It allows one to quantify the degree of similarity between structures. Provided standards are known (i.e., a few molecules with known properties are taken as leads), other molecules in the group can be ordered (partial ordering will result in general, i.e., hierarchical, ordering, in which some compounds can be ordered, while others are not comparable).

Given a collection of compounds of which a few are known to be toxic, others can be ranked, and those found sufficiently similar to toxic compounds can be identified. Equally, if a compound is

compared with a few known nontoxic compounds and found to be similar, one can expect the compound to be nontoxic.

This approach requires information on apparently similar structures. At present, no explicit recognition of heteroatoms is taken into account; the approach needs to be extended, but can be used in the present form if heteroatoms are in similar locations. A recent advance involves extension to include weighting procedures. This appears to make the approach potentially more powerful in more than one direction (e.g., by focusing on strategic fragments).

In summary, graph theory provides a framework for comparison of structures. For different applications, it seeks the most relevant fragments; it has potential for extension beyond connectivity (inclusion of heteroatoms). Its input is the information on molecular structure, and its output is a ranking of structures — by their similarity to the chemicals selected as standards. Graph theory tells us what may follow from given connectivity; it does not enter into details of mechanism. If two molecules are graph-theoretically similar, graph-theoretical schemes suggest that they also have some physical, chemical, and biological similarity.

Choosing Among Predictive Models

If the chemical compound being assessed for toxicity is a member of a suitable structural class, there may be predictive mathematical models that are available or can be developed. Each model corresponds to one biological activity, is derived from a specific set of compounds, and uses a specific set of descriptors. Before using the model to make a prediction, one must assess the degree of similarity between the compound and the model compound set. This can be done, based on the model descriptor set, by using multivariate statistical methods such as Mahalanotis distance and Wilks' lambda. These multivariate statistical methods give measures that are F-distributed. Thus, one can make statistically based statements about the degree of similarity between the compound and the model set of compounds.

Recommendations

Development of model-generation methodology should be supported.

Data sets suitable for analysis by these methods must be developed systematically.

3. THEORETICAL STRUCTURE-ACTIVITY STUDIES USING CAUSALLY RELATED MOLECULAR PARAMETERS

The evaluation of large numbers of diverse chemicals for a spectrum of potential toxic effects is an enormous task if those chemicals that could be industrial and environmental agents are included. Structure-activity studies based on computational chemistry can be very useful in such evaluations. All computational methods require at least an initial data set to test the reliability of selected molecular descriptors prior to their use in predictive screening. The theoretical methods have been developed to use such experimental data to this end.

A major obstacle to the assessment of new and existing chemicals is the lack of experimental data relevant to evaluating toxic effects. Useful data must include more than single-dose acute whole-animal toxicity measurements. Specifically, information is needed from systematic and controlled studies on (1) effects of compounds on a variety of isolated cell types, (2) effects of compounds on biochemical processes in tissues, (3) mechanisms and products of biotransformations, (4) properties of metabolites, (5) long-term toxicity, including time-dependent discrete analysis of lesions and effects induced by the chemicals, and (6) physicochemical properties, such as partition coefficients and pK_a values.

Because of practical considerations, it is impossible to obtain data in this detail for all potentially hazardous classes of compounds. Thus, a five-step protocol is suggested, as shown in Figure 1. The first step is to divide the chemicals into subgroups according to structure and functional groups by any one of a number of procedures; the simplest is a "chemical reactivity" group categorization.

The next suggested step is selection of compounds from each class for a series of assays for adverse effects such as acute toxicity, mutagenicity, and carcinogenicity; this provides an experimentally determined structure-activity data set, i.e., a "data base." The more specific the biological activity tested, the more useful the tests will be in relating structure to activity. For example, if a given toxicity can be related to inhibition or activation of a specific enzyme or to chemical modification of a nucleic acid or protein component, molecular properties related to the toxicity will be more apparent.

As a third step, mechanistic studies are suggested for a very limited number of representative compounds of different classes of chemicals that have been tested and found to produce any toxic effect of interest. Any information from such studies that gives an indication of the origin of the adverse effect can be very useful in selecting mechanistically relevant molecular descriptors. While such in-depth studies might appear to be relatively time consuming, they should ultimately lead to more effective and rapidly converging schemes for obtaining reliable molecular descriptors that also give insights into mechanisms of action.

Once chosen, each set of candidate molecular descriptors must be obtained by methods ranging from direct experimental determination to purely theoretical calculation (step 4 in Fig. 1). The reliability of the calculated molecular descriptors in accounting for known activity can then be tested by using the available experimental data (step 5). If tests are successful, the use of the set of descriptors for large-scale screening is indicated; if tests are not successful they should be re-examined for their mechanistic relevance and for the validity of the procedures by which they were obtained.

The techniques of theoretical chemistry can be particularly useful in three different steps of the process outlined in Figure 1: (1) elucidation of mechanisms (step 3), (2) selection of molecular descriptors mechanistically relevant to a specific risk (step 4), and (3) calculation of selected molecular descriptors of potential use in screening large numbers of molecules (step 5).

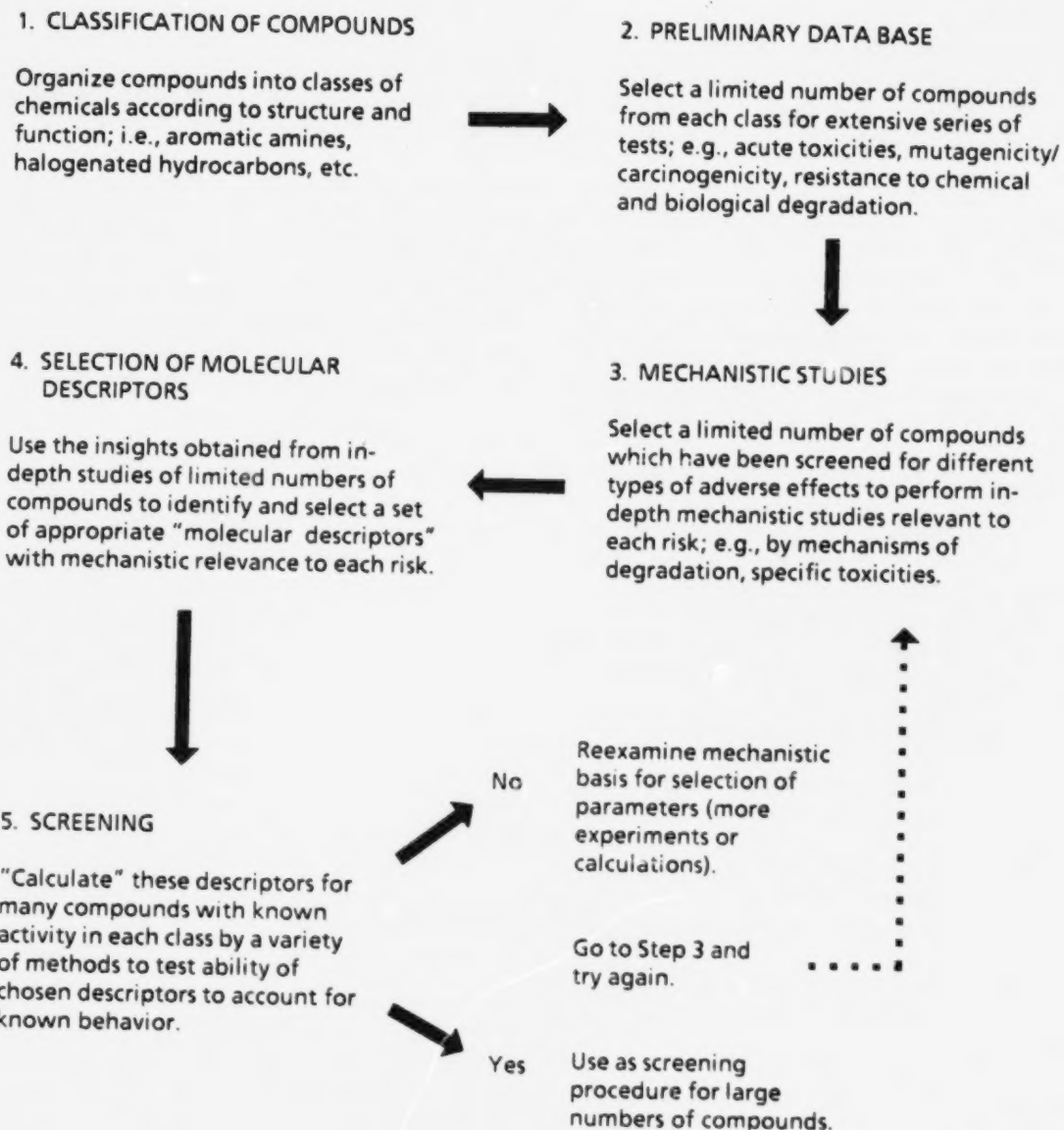


Figure 1. Five-step protocol for using SAR methods to evaluate large numbers of chemicals for biological effects.

Any knowledge of the mechanism of action can be very helpful in determining which molecular properties are most likely to correlate with toxicity. However, it should be stressed that a detailed understanding of the mechanism of what is often a complicated interaction, taking all relevant factors into account, is not a prerequisite for developing, by a theoretical approach, a useful SAR. This relationship can then provide further information on the mechanism of action.

The inherent capabilities of theoretical chemistry are embodied in a hierarchy of computer programs and methods. These techniques can essentially characterize (1) individual molecular systems, (2) complex formation between individual molecular systems, and (3) chemical reactions between molecular systems. These capabilities render computers the "laboratory" where chemical reactions, complex formation, and properties of individual molecular systems can be modeled and calculated. The techniques used are not limited to one particular method. Figure 2 schematically indicates molecular events that could lead to a toxic response. All these actions can be ascribed to the physical and chemical properties encoded in the molecular structure of the agents and the responses they evoke in the biological systems.

Calculation of Molecular Properties of Individual Compounds

The following calculated properties can be used to evaluate and predict toxicities:

- A. Structural properties
molecular geometry
conformational energy, free energy
a set of accessible conformers
- B. Electronic properties
net atomic charges
electron density distributions
dipole and higher moments
partition coefficient (hydrophobicity/hydrophilicity)
ionization potentials
electron affinities
molecular electrostatic potentials and electric fields
- C. Chemical reactivity properties
estimates of group or atomic electrophilicities and nucleophilicities

These calculated properties can be useful in two ways: (1) to serve as a basis for analogue comparisons relating chemicals with unknown toxicity to those with known toxicity and (2) to provide candidate molecular indicators of toxicity for all levels of existing information on toxic effects, from an initial systematic data base to detailed mechanistic studies.

In regard to the first use, these properties can be used to evaluate similarities among compounds in order to relate a variety of structures to similar activities. For example, given molecules of similar structure, how similar are the molecular properties that might be responsible for recognition, specificity, and reactivity? Also, given that a particular physical property, or properties, can be established to be the determinant for recognition, specificity, or reactivity, can two structurally dissimilar molecules be conformationally altered to become functionally similar by adopting conformations in which their determinants for recognition are made to coincide?

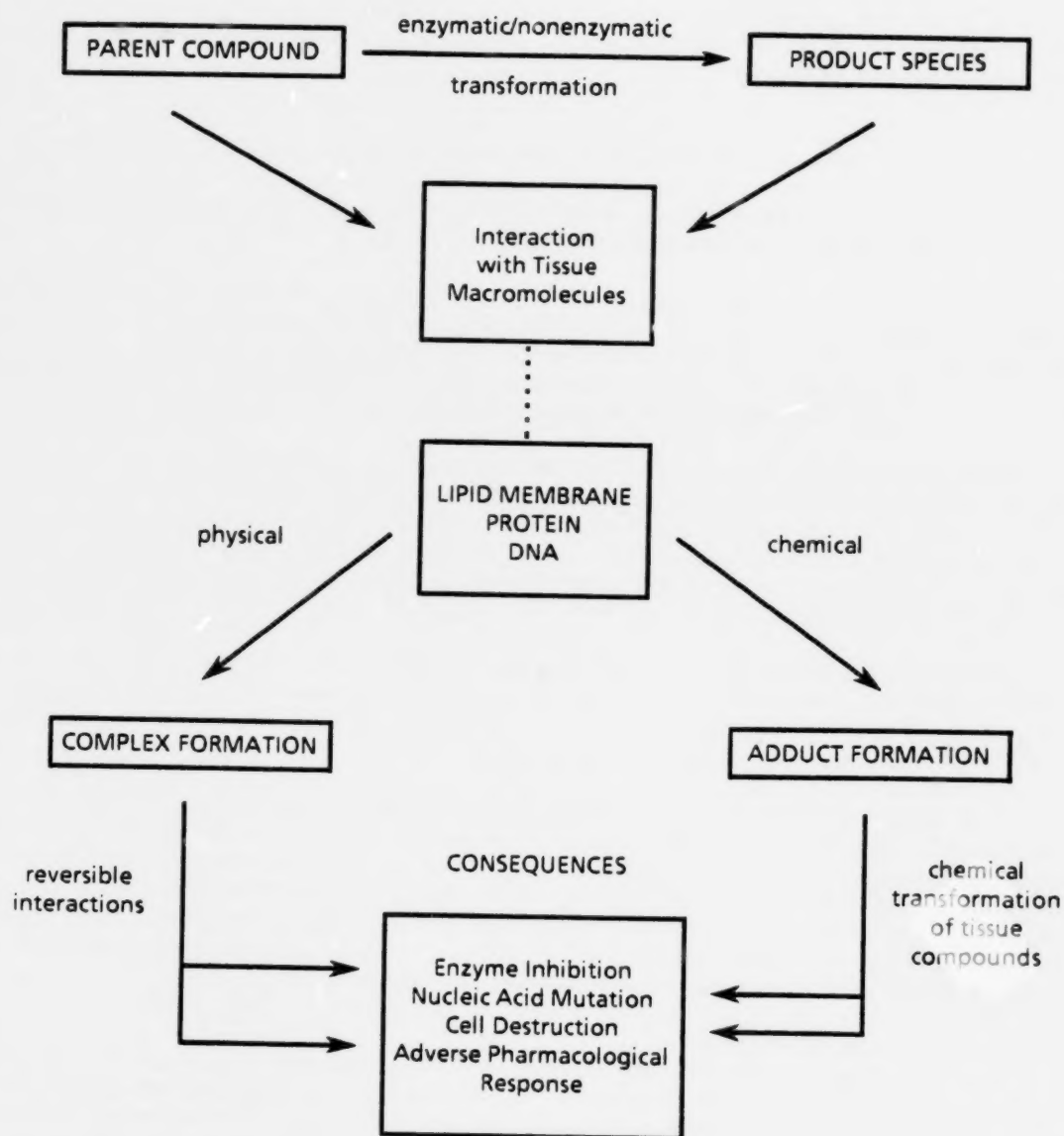


Figure 2. Molecular events that could lead to a toxic response.

In regard to the second use, calculated structural and electronic properties can be tested systematically for their ability to predict relative potencies for a given end point. Mechanistic inferences can be made from those indicators that successfully predict activity. The use of these properties requires the application of techniques based on structural superposition, either of rigid molecules or of those with conformational flexibility, in addition to the computation of the molecular properties of the various structural forms. Some of the methods used for structural superposition are outlined below.

Structural Properties--

A number of types of methods exist for computing molecular geometry and conformation based on energy minimization. Depending on the size and number of degrees of freedom and the degree of accuracy required, empirical energy methods, semi-empirical quantum mechanical methods, and *ab initio* quantum mechanical methods can be used.

Empirical energy methods rapidly minimize the energy of a molecule with respect to all degrees of freedom. A major problem with flexible molecules is determining which of a number of stable geometries is responsible for the toxic effects. In addition, the presence of many stable conformers makes comparisons among flexible molecules particularly difficult. Methods for making these comparisons are available, but they require a sampling of conformational space.

Molecular dynamics provides one method for exploring the conformational space of a molecule. This method yields the time course of conformational fluctuations and transitions of a molecule. This evaluation can be monitored directly on an interactive graphics display, and minimized energies can be calculated from configurations found at a number of points along the trajectory. Alternatively, initial configurations can be obtained by systematic variation of conformational parameters.

Energy minimization is also useful in superposition of flexible molecules. We can compare structures and conformations quantitatively by requiring common or equivalent atoms to superimpose while minimizing the intramolecular energy. In this way, we can see how well two molecules can superpose and at what cost in energy.

Both semi-empirical and *ab initio* quantum mechanical methods for calculating optimized structures provide a consistent basis for relating molecular structure and molecular properties because only quantum mechanical methods can be used to calculate electronic and reactivity properties. These methods are used both for exploring the full conformational space and for providing a detailed structural description of selected minimum-energy structures. These are the only methods that can be used to calculate structures of molecules that contain atoms or groups for which parameters are not available in empirical energy formulations.

Electronic and Reactivity Properties--

Both semi-empirical and *ab initio* quantum mechanical methods can be used to calculate electronic properties related to the compounds' abilities to undergo enzymatic and nonenzymatic transformations and to interact with biological components that leads to (1) reversible complexes (e.g., receptor-drug or enzyme-inhibitor complexes), (2) covalent adducts, or (3) irreversible chemical change in cell components (e.g., radical formation).

Among the properties of individual molecular species that could be relevant to these processes are electronic properties directly obtained in a self-consistent manner at the same time optimal energies are obtained. These are net atomic charges, electron density distributions, ionization potentials, and electron affinities (in the approximation that the latter two can be estimated as the energies of the highest-energy-occupied and lowest-energy-empty orbitals). Also directly available from the calculations are measures of chemical reaction, the frontier orbital (i.e., superdelocalizability) parameters for estimating nucleophilic and electrophilic sites of attack in given molecular species. These parameters can be used to predict sites and infer mechanisms of

biotransformations and covalent interactions with tissue macromolecules. In addition to such direct properties, calculated optimized molecular structures and electron density distributions can also be used to calculate dipole and higher moments and molecular electrostatic potentials as additional measures of these processes, particularly of reversible interactions with key biological components.

The electrostatic potential is a quantitative calculation of the initial interaction between a molecule and an approaching chemical species. It is one of the most powerful descriptors of the molecular properties that determine chemical reactivity, and it provides information on both the magnitude of interactions and the likely relative orientation between the two interacting species. It has been found that certain features of the electrostatic potentials of a given class of molecules can often be related to the molecules' degree of activity in specific biological tests. For instance, it has been shown that the most negative values of the electrostatic potential near the oxygen atoms of halogenated epoxides are good indicators of their carcinogenic activities.

The molecular electrostatic potential has been used many times to explain and predict the activity of chemicals and drugs in biological systems. The application of the electrostatic potential can be illustrated in relation to the toxicities of dioxins. By computing potential maps for dioxins of known toxicities, one could identify key features that correlate with the biological activity. The toxicities of untested dioxins could then be predicted by computing their potentials and comparing the key features with dioxins of known activities. This is a kind of reasoning by analogy, but the analogies are to computed properties. Similarly, the toxic portions of molecules (their toxicophores) could be identified and compared in terms of their electrostatic potentials.

Characterization of Intermolecular Complexes

As more detailed information becomes available from experimental or theoretical studies, it becomes feasible to characterize the stereochemical features of complexes formed between parent or transformed chemical species and key biological components.

In instances in which the active species and/or the target molecule can be inferred, at least key portions of an interactive complex can be characterized. In such cases, techniques exist to calculate optimum complex geometries and relative binding energies. For example, empirical energy methods have been used to characterize enzyme-inhibitor complexes and model drug-receptor interactions. These results led to an understanding of the key features in the recognition at biological targets and of the mechanisms ensuing from the formation of the complexes. This information constitutes a resource for the development of methods and parameters to be used in the prediction of toxicity. Vigorous research in this area is therefore necessary to ensure a rigorous basis for the analysis and prediction of toxicity and to make possible the development of efficient and accurate computerized methods to carry out such analyses and predictions.

The use of empirical energy methods with interactive graphic display systems to help execute "docking" experiments (in which the approach of a chemical toward its site of action can be modeled and observed on a graphics terminal) is potentially the most powerful method for assessing activity, e.g., inhibition or toxicity, when the necessary information exists. An example of a presently known receptor structure of interest is DNA. An extremely productive direction for the U.S. EPA to consider is the structural determination of systems such as the Ah-protein-dioxin system, azoreductases, and the NTE protein. Other systems for which targets can be identified and are expected to be relevant to a significant number of future compounds submitted should also be candidates for such an approach. These studies are not trivial, but certainly are within the capabilities of current technology. Techniques involved include protein purification or, alternatively, isolation, cloning, and expression of the gene to produce the protein. Knowledge of target structure is one of the most significant pieces of information that can be obtained when attempting to understand structure-activity relationships.

Interactive Graphics--

The use of three-dimensional interactive graphics systems to compare molecules, to gain an appreciation for their structural and functional properties, and to compare such features by superposition and manipulations of the molecules is a powerful and efficient way to assess relationships between molecular properties and biological interactions. This technique can also be important in evaluating the degree of similarity of molecules with similar properties or activities and in the development of data bases containing such information.

Chemical and Biochemical Transformations

The techniques of theoretical chemistry — on the semi-empirical and *ab initio* quantum mechanical levels — now permit the characterization of chemical reactions. This description includes the calculation of optimized geometries and electronic structures of reactants, intermediates, and products as well as transition states, thermodynamics, and kinetics of reactions.

As mechanistic information becomes available on the biological actions of a few selected molecules, including biotransformations, interaction of source materials with environmental components, and chemical interactions with known target biological molecules, parallel theoretical studies can be made on these chemical reactions. The information obtainable from such studies can (1) help identify intermediates that would be too transient to be characterized experimentally but that could contribute to a toxic effect and (2) help identify new molecular indicators of parent compounds and intermediates that could be reliable indicators of the toxic effects.

While it is not practical to characterize chemical reactions as a part of large-scale screening, it is possible to do such studies for selected analogues and to use the information to identify and calculate individual molecular properties that are appropriate and cost-effective to use in large-scale screening. Examples of possible reactions that can be modeled are (1) model enzyme-substrate reactions, (2) nonenzymatic transformations such as isomerization, hydrolysis, and radical reactions, and (3) reactions between source compounds and environmental components.

Ab Initio Calculation of By-product Formation

We recognize a need for identifying possible secondary pollutants, that is, molecules that are formed as by-products in industrial and environmental reactions and that may have toxic effects. These include various nitrogen and sulfur oxides, trace organic compounds produced in the atmosphere (e.g., propylene), and by-products of combustion processes. The first need is to determine which molecules are the most likely by-products in various processes; the second need is to predict their degrees of toxicity.

Determining the molecules that are likely to be formed will require the calculation of energy surfaces and activation energies. In many instances, especially when radicals are involved, highly sophisticated quantum mechanical computational techniques, such as configuration interaction methods, will be needed for the calculations. While there are well-established procedures that can be used for this purpose, they are at a more complex computational level than the methods that are normally used for predicting biochemical behavior and toxicity. One consequence of this is a limitation on the sizes of molecules that can be studied and the need for specially trained scientists to carry out such studies.

4. APPLICATION POSSIBILITIES FOR ARTIFICIAL INTELLIGENCE DECISION AIDS IN THE EXISTING PMN REVIEW SCENARIO

Artificial intelligence (AI) methods such as expert systems work best when applied to well-established, repetitious reasoning problems such as the Premanufacture Notification (PMN) review, which has been carried out weekly for the past five years. This process as we understand it is outlined below with a numerical index that ranks the difficulty of building computer aids for each step. The difficulty scale is 1 to 10; trivial = 1 and impossible (with current methods) = 10. We assume that the most appropriate computational method, which is not always an AI approach, would be used at each step. About 25 compounds are considered per week.

I. Decide strategy for handling each compound.

- (a) Unique chemistry--use whole molecule.
- (b) Functional groups--form analogue sets based on reactivity, steric factors, etc.
- (c) Put obviously nontoxic compounds in a special bypass group.
- (d) Put obviously toxic compounds in a special attention group.
- (e) Put polymers in the bypass group unless obviously toxic.
- (f) Treat structural series as a single problem.
- (g) Schedule review process to meet regulatory deadline of 90 days.

A decision-aid expert system that analyses the molecule should be able to make this strategy decision (probably a production rule system). (Difficulty factor = 5.)

II. Search data base on Structure and Nomenclature Search System (SANSS) (10 compounds per week).

- (a) Check results from previous PMN molecule searches for appropriate examples.
- (b) Form search query based on composite template for simulant analogue set based on consideration of reactivity and metabolism (see section on metabolism).
- (c) Do successive searches; relax (or strengthen) similarity requirements between runs until successful.*
- (d) Choose analogue simulants to be researched further.

An AI natural language front end to the data base management system and decision rules for forming templates and recognizing success could help this process. (Difficulty factor = 6.)

III. Search data bases for biological data on specialty files using CAS number from SANSS search.

- (a) Choose data base of special interest (Toxline, inhouse data, PHS149, etc.).
- (b) Choose general-interest data bases (Chem Abstracts, CBA, DARC, etc.).
- (c) Carry out searches as in II.
- (d) Omit patents.
- (e) Check local archive of previous PMN compounds.
- (f) Compile list of literature references for library search.

*There exist today computational functions that can measure similarity between two structures, as noted later in the section on data bases. Such functions could be used to select analogues from the inhouse data base of compounds, and they can retrieve similar structures that have more diversity than could be retrieved by standard substructure search, even with generalized queries.

A system related to II but generalized to handle translation of search queries to other data base conventions should work. (Difficulty factor = 7.)

IV. Search literature using various libraries in Washington.

- (a) Check microfilm from previous PMN search.
- (b) Check Library of Medicine, etc.
- (c) Read references and do concept extractions on biological results and physical data.

This would require a page reader such as that used by the blind at the Library of Congress to put text into computer-readable form. Figure analysis, particularly for structures, would require scene-analysis AI systems. Concept extraction from text is a computational linguistics area of active research. It is very difficult in unconstrained domains; however, the biological and chemical literature does have certain linguistic constraints. (Difficulty factor = 9.)

V. Run CHEMEST program from Arthur D. Little Corp. to calculate physical properties of PMN compounds.

- (a) Compute all options available.
- (b) Check archive of previous runs.
- (c) Compile list of best estimates on the properties.
- (d) Determine biological and ecological hazard influence of the parameters, particularly for the obvious bad actors.

An expert system to prepare input for a conventional program and to analyze the results is quite reasonable. (Difficulty factor = 6.)

VI. Set up the Structure Activity Team (SAT) meeting and assign researchers to evaluate the data found on the simulant analogues. Experts are required in the following:

- (1) general toxicology
- (2) environmental toxicology
- (3) environmental chemistry
- (4) chemical carcinogenesis
- (5) chemical mutagenesis
- (6) pharmacokinetics
- (7) QSAR

- (a) Line up panel of experts.
- (b) Retrieve minutes of meetings in which related compounds were discussed and the decisions were made on those compounds.

If certain experts are unavailable for the meeting, one might have a series of expert systems available, each with a knowledge base in one of the above specialties. One of the other committee members could run the program to see if any red flags appeared based on the biological and physical data for each of the ten compounds reviewed that week. The expert system results might also be used as a second opinion at the meeting. (Difficulty factor = 8.)

VII. Conduct SAT committee meeting.

- (a) Check the bypass compounds again for special obvious problems.
- (b) Discuss the researched compounds.

- (c) If previous, related PMN decisions are applicable, use them again, and be sure that the justification given in those decisions reflects current knowledge in each of the fields.
- (d) Each committee member acts as a devil's advocate relative to the compound's influence on people and the environment in his or her area of expertise.
- (e) Classify into potentially toxic or insignificantly toxic classes and identify toxicity action modes and target organs, if possible. Action modes include carcinogenicity, mutagenicity, reproduction toxicity, nervous system toxicity, etc.
- (f) Document the decisions and justification.

Computer automation of this step is outside the area of current capabilities. Although some unconventional ideas could be stated, they would detract from the validity of the rest of this analysis. (Difficulty factor = 10.)

VIII. Update the inhouse data bases and archives (Difficulty factor = 5.).

- IX. Combine the findings of the SAT committee on hazards with those of the Exposure Analysis Meeting (EXAM) to decide if special handling is required for the compound and if manufacture will be permitted.

Although we have not been briefed on the EXAM procedure, it seems that evaluations might also be computer aided somewhat. The Oak Ridge National Laboratory as well as the Japanese are currently using AI systems for safety engineering assessment of chemical processes. (Difficulty factor = 9.)

Expert Systems Approach

Capturing the expertise of toxicologists on the PMN panel and elsewhere would be useful; a knowledge engineer could begin this task by observing panel meetings to develop rules and analyzing past minutes of these meetings. The agency is in the unique position of already having experts meeting regularly, so the only additional expense would be the cost for the knowledge engineer and, perhaps, for consultation with knowledge engineering experts. The expert system could be built over time, operating in parallel with the panel, so that results could be evaluated and improved.

In the above analysis, reasoning by analogy from examples is required at several steps, particularly in selecting analogue simulants and drawing conclusions about the applicability of the biological activity and physical properties found in literature references for the simulants to the PMN compound. The logical connection of experimental measurement and activity prediction is particularly important in establishing credibility for the predictions. Reasoning by example is easy for scientists to follow and has the advantage of linking the compound for which there are few data to compounds for which there may be many data. It can be effective even if there is only one analogous compound and thus is useful for situations in which statistical methods are not well suited. Contemporary expert systems do not have reasoning-by-example or reasoning-by-analogy capabilities, and this is an area that should be developed through research funding.

Metabolism Expert

Analysis of the role of metabolism in activation of compounds can be assisted by an expert system such as the XENO program developed at the University of California at Santa Cruz. Mechanisms of metabolism are represented in a biotransform library. Mechanisms are automatically selected and applied to the problem as appropriate. A "tree" of plausible metabolites can be generated for consideration by experts. The advantage of computer analysis of metabolism is that one is assured that no possibilities are overlooked, even when multiple-step metabolism is involved. The technology already exists, but additional work is needed to extend the library of metabolic mechanisms and to provide for transport phenomena, such as excretion, via log P estimation. Each

metabolite generated could also be analyzed for potential biological activity by an expert module and/or could be searched for in the inhouse data base.

Data Base Requirements

The reasoning-by-example approach rests on the availability of a suitable data base. A high priority must be the development and maintenance of large, relevant, and searchable data bases. The data bases must contain compounds of little or no toxicity as well as active compounds; the data bases must be searchable by full chemical structures and substructures and, in addition, by other structural and physicochemical properties. These data bases would serve several purposes. They would capture the record of chemicals considered, analogues considered, and links to the primary literature containing toxicology data used.

Searching

Given the existence of the data base, the software used to search it should be the best available. Substructure searching is the starting point for finding similar structures. The structures can be trimmed by consideration of physicochemical properties. Various new techniques for searching are now emerging, and they should be evaluated for application to toxicity assessment.

Structural Similarity Assessment

The degree of structural similarity between the chemical whose toxicity is sought and the structures for which toxicity is found must be assessed. A number of methods have been developed for quantitatively expressing the degree of similarity between compounds. Many are based on graph-theoretical approaches to molecular structure. Atom pairs, path counts, and molecular connectivity indices are examples of topological representations that have been used for this purpose.

Much more generalized definitions of similarity can also be generated. These would be based on other classes of descriptors beyond topological representations. Examples of such descriptors that could be useful in this context include molecular properties such as three-dimensional structure, conformation, and the resulting electronic properties (see section 3). The descriptors employed are open ended because this is an active research area.

5. THE NEED FOR A TOXICITY DATA SET DESIGNED FOR SAR ANALYSIS

The rate-limiting factor in developing better mathematical models for the correlation of chemical structure with biological activity is the quality and quantity of the toxicity data. Hence, a significant recommendation is that the U.S. EPA sponsor a project to obtain first-class toxicity data in a well-defined system for a very carefully selected set of organic compounds.

Chemicals that can be treated by the various SAR models now being studied should be selected; they should provide two kinds of variability:

- (1) The set of compounds should include subsets of congeners of 20 to 50 compounds that presumably produce an observed biological response by the same mechanism. There should be general agreement that the structural variations in the set are as broad as possible, yet not so gross that they cannot be dealt with by techniques ranging from simple QSAR to quantum chemistry.
- (2) The second (and more difficult) type of variation that should be included in the main data set is differences in the toxicophores that, nevertheless, produce the same qualitative type of biological response. This is the most difficult type of SAR analysis, and this type of data is vital to the use of pattern recognition and the other general approaches to SAR.

One could debate endlessly over which biological test should be selected. However, for such a study to be of value, several hundred chemicals must be carefully tested; this means that the test must be relatively simple and inexpensive. It must also be a test in which specificity plays an important role. The problem of accounting for rather large changes in biological response resulting from relatively small changes in structure presents the greatest difficulty to the various methods now used for relating chemical structure to biological activity.

For testing and developing the various SAR methods, the type of biological end point chosen is not as important as its cost. If we can correlate one truly complex type of biological activity with chemical structure, the evidence in hand suggests that we will be able to correlate many. Hence, an Ames test seems a logical choice. Although there is disagreement on whether it is an ideal model for carcinogenicity, almost everyone could agree that it does have practical importance. It shows great variation, on the order of 10^8 , with various chemicals, and some degree (but not too much) of metabolic activity is involved. The test is so widely employed, both in industrial and academic laboratories, that any mathematical models developed would automatically be tested by many different types of laboratories all over the world.

We have been informed that a scientific study of the effectiveness of the current PMN evaluation procedure is planned. This study would involve 100 chemicals chosen from those submitted to and acted upon by the OTS/SAT team. The study would involve various relevant experimental end points. We recommend that this study be performed and that its results be made available to the scientific community. These data will help evaluate and improve methods to predict toxicity, increase our understanding of the mechanisms of toxicity, and suggest possible ways to improve the PMN process.

APPENDIX A



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

SEP 18 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Dr. James Rabinowitz
U.S. Environmental Protection Agency
MD-68 (Bldg. ERC, Rm. K-206)
Research Triangle Park, N.C. 27711

Dear Jim:

This letter concerns a specific need of the Office of Toxic Substances for the assessment of carcinogenesis/mutagenesis hazard of new chemicals submitted for evaluation as a PMN. The oncoming QSAR Workshop that you are organizing will bring together many talents looking at QSAR from different angles. Maybe one of the problems which could be addressed is this particular need of OTS. This could greatly help some of our hazard assessment problems. So here it goes.

We receive in the PMN program a number of polyazo dyes which, when structurally intact, should by structural criteria be non-mutagenic and non-carcinogenic. However, the submitted compounds often contain a substituted benzidine or amino-naphthalene moiety which may (and can) be liberated when the intact molecule is split by azo reductase action such as present, for example, in the mammalian liver or in colon bacteria.

Understanding is very scanty on the steric and electronic parameters which govern the susceptibility of an azo double bond to azo reductase attack. The typical mammalian and bacterial azo reductases attack azo linkages between two aryl groups. As far as I know these azo reductases do not attack azo linkages between two aliphatic moieties, and I am unaware if the susceptibility of an aryl/alkyl sandwiched azo linkage is known. Moreover no systematic study has ever been carried out on the electronic/steric effects of various substituents on the molecular moieties, on both sides of the azo linkage, in influencing susceptibility to azo reductase attack.

In assessing the potential carcinogenicity/mutagenicity hazard of such polyazo dyes we have - in the absence of data - no choice but to take the conservative approach and assume that the potentially mutagenic/carcinogenic moieties built into these structures are liberated at a high rate by azo reductase attack. However, it is of course probable that substituents may

considerably affect the susceptibility of the linkage to azo reductase, and some azo linkages may be quite stable. Thus, the study of the conditions which influence the susceptibility of azo linkages to azo reductase attack is of great importance to the Agency and to the industries which may be affected by possible regulatory actions on such compounds.

Hoping that some of the participants may be able to address and possibly help with this problem, I am,

With best personal regards,

Sincerely,

Joseph

Joseph C. Arcos, D.Sc.
Senior Environmental Health
Scientist, US EPA
(Adjunct) Clinical Professor,
Tulane University Medical Center,
New Orleans

APPENDIX B

TOXICITY ASSESSMENT AND THE USE OF SAR UNDER SECTION 5 OF TSCA

Note

The discussion that follows was originally prepared for another audience in EPA, and the term "structure-activity relationships" (SAR) refers to the techniques used by the EPA Office of Toxic Substances in evaluating the potential toxicity of so-called "new" chemicals submitted to EPA under section 5 of the Toxic Substances Control Act. The approaches employed in EPA's applications of SAR differ to a substantial degree from the approaches typically associated with the use of quantitative structure-activity relationships (QSARs) and other computational techniques to predict the biological chemistry of xenobiotic substances. The chief constraint on the use of QSARs and other computational techniques by the EPA at present is that the problem confronting EPA differs from the earlier formal applications of these techniques (for example, in the pharmaceutical industry) in two important ways. First, the desired goal (prediction of adverse health effects) is not a single endpoint, but depends on multiple interactive paths, and the mechanisms involved are often not well understood. Second, there are few experimental data available or easily obtainable, not only relative to health effects of the PMN chemical, but also relative to all but the simplest physical chemical properties. Further, the chemicals confronting EPA are often not the simple extension of a family of similar chemicals where a great deal of data exist for other family members (as is often the case in pharmaceutical applications of these techniques).

INTRODUCTION

The Toxic Substances Control Act (TSCA) was passed by the Congress and signed by President Ford in 1976. The stated purpose of the Act is to "protect human health and the environment by requiring testing and necessary use restrictions on certain chemical substances" (P.L. 94-469, 1976). Under TSCA, the Congress decided to distinguish between so-called "new" and "existing" chemicals.* New chemicals, that is, those not appearing on an inventory of existing chemicals, are subject to premanufacture reporting requirements under section 5 of TSCA. Since publication of the inventory of existing chemicals in July 1979, Premanufacture Notifications have been received on some 4000 new chemicals (see Figure B-1).

TSCA section 5 requires that manufacturers and importers of new chemicals submit a Premanufacture Notification (PMN) to the EPA 90 days before they intend to commence manufacture or import. TSCA thus provides EPA with a 90-day review period, extendable with cause to 180 days, in which to complete its risk determination on each PMN chemical. (The task before EPA in regulating chemicals under TSCA is to distinguish between "reasonable" and "unreasonable" risks; as used in TSCA, "risk" is some function of hazard, i.e., toxicity, and exposure, and includes

*Pesticides, drugs, foods, food additives, cosmetics, and certain other chemicals which are controlled by other statutes are not within the purview of TSCA (see U.S. Code, Title 15, Section 2602 (2)).

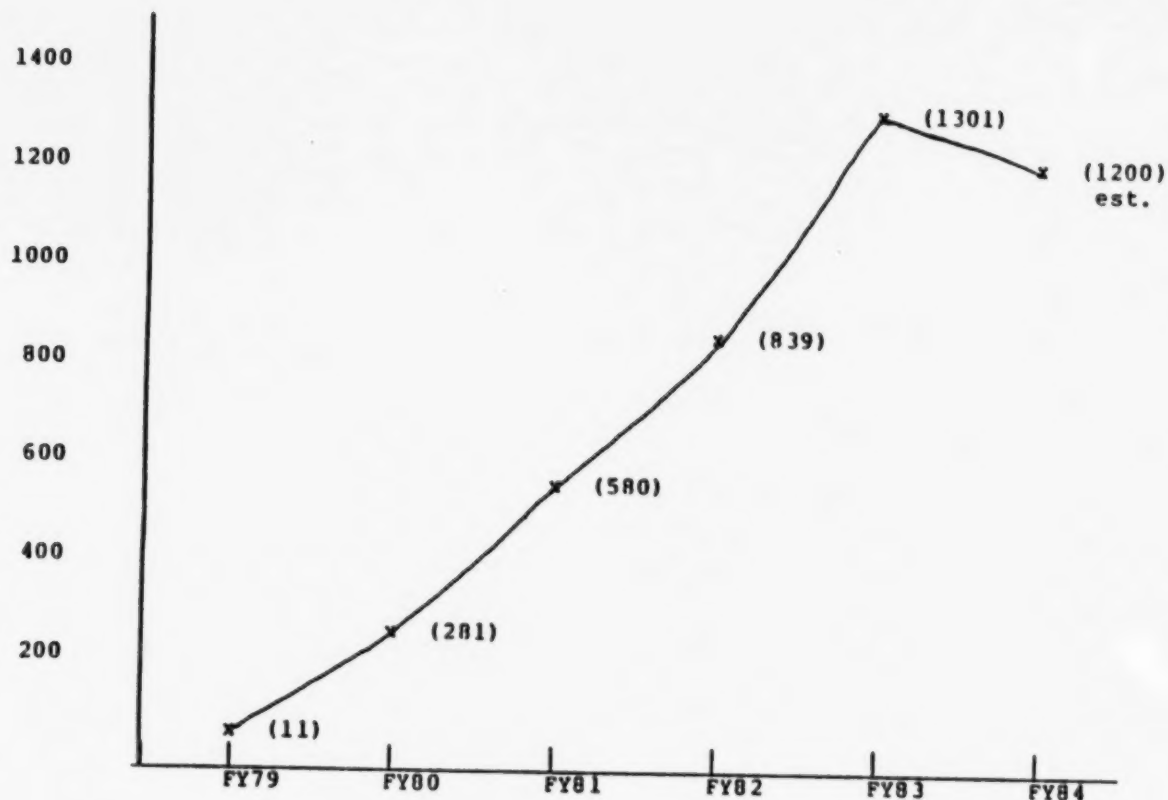


Figure B-1. Annual receipt of PMNs from fiscal year 1979 through fiscal year 1984 (EPA, 1984b). FY = fiscal year.

consideration of economics.) Under TSCA section 5, certain information must be provided in the notification, as follows:

- a description of the new chemical substance, including its chemical identity, molecular structure, and common or trade name;
- the estimated total amount to be manufactured or processed;
- the proposed categories of use and the estimated amount to be used for each category;
- a description of the by-products resulting from manufacture, processing, use, or disposal;
- the number of individuals expected to be exposed in their place of employment and estimates of the expected duration of exposure;
- the manner and methods of disposal; and

- any test data in the possession or control of the notifier related to the health or environmental effects of the substance (15 U.S.C. 2604(d)).

As can be seen in the above listing, TSCA does not require that submitters conduct toxicity testing prior to submission of the PMN; rather, they need only supply any health or environmental test data available to them at the time of submission. Presently, EPA receives test data on fewer than 50% of all PMNs submitted; when provided, the data most commonly consist of acute lethality and local irritation studies (see subsequent discussion on this point).*

Given the general paucity of submitted test data on PMN chemicals, the EPA Office of Toxic Substances has come to rely on "structure-activity relationships" (SAR) in its evaluation of the potential hazards posed by these chemicals. The analysis performed by EPA in its use of SAR involves the following components:

- review of submitted test data, if any, on the PMN chemical;
- review of test data available on structurally analogous substances (these can be identified by either EPA or the submitter);
- use of quantitative SAR methods where available and applicable; and
- the professional judgments of scientific assessors in interpreting and integrating the above, along with consideration of factors derived directly from the structure of the PMN chemical. These factors can include, for example, molecular shape and size, fundamental physical/chemical properties, log P, presence and positioning of reactive chemical functional groups, metabolic pathways, and so on (see Arcos, 1983; Arcos and Auer, in press).

*The TSCA "premanufacture" reporting requirements can be contrasted with the European Communities (EC) "premarketing" notification requirements (EC Directive 79/831/EEC, 6th Amendment, 1979). As the terms indicate, premanufacture notification under TSCA is required at an earlier point in the development of a chemical than is the case for the EC's premarket notification procedure. Many of the information-reporting requirements under the EC's directive are similar to those outlined earlier in TSCA, with the major difference being that the EC Directive (1979) requires as a mandatory part of the premarketing notification a specified "base set" of health, environmental, and physical chemical test data. A minimum set of test data is thus available on premarket notification (EC) chemicals, whereas the hazard assessment of TSCA premanufacture chemicals often starts out with fewer or no data. The EC Directive specifies the following set of testing requirements: Physical Chemical Properties: melting point, boiling point, relative density, vapor pressure, surface tension, water solubility, fat solubility, partition coefficient, flash point, flammability, explosive properties, auto-flammability, and oxidizing properties; Toxicological Studies: oral LD₅₀, inhalation LD₅₀, dermal LD₅₀ (routes(s) for administration will be selected based on physical chemical properties and intended use), skin irritation, eye irritation, skin sensitization, 28-day subacute toxicity study (dosed 5 to 7 days per week for at least 4 weeks with route selected on basis of intended use, acute toxicity, and physical/chemical properties), and mutagenicity (2 tests, one bacteriological, with and without metabolic activation, and one non-bacteriological); Ecotoxicological Studies: acute LD₅₀ (fish and daphnia) and degradation (biotic and abiotic).

The task before EPA is to determine, despite a paucity (often times an absence) of test data, whether PMN chemicals, under their projected conditions of use, manufacturing, or processing, "may" or "will" present an unreasonable risk of injury to health or the environment. Under the former (TSCA section 5e, or "may present") finding, the Agency can prohibit or limit manufacture, processing, use, or disposal of the new chemical pending development of test data sufficient to permit a reasoned evaluation of the risk posed by the chemical (U.S. Code, Title 15, Section 2604 (e)). In the event that EPA can support the finding that a new chemical "will present" an unreasonable risk, the Agency can take action under TSCA section 5f to prohibit manufacture altogether or to limit its use or release without a requirement for development of additional test data (U.S. Code Title 15, Section 2604 (f)). To date, EPA has issued two TSCA section 5f orders (involving three PMN chemicals), and seven unilateral TSCA section 5e orders (involving 15 PMN chemicals); 23 voluntary or consent 5e orders (involving 150 PMN chemicals) have also been instituted.* In addition, 77 PMN chemicals have been voluntarily withdrawn by the submitter in the face of a likely TSCA section 5e or 5f order (EPA, 1984a).

In the event that EPA chooses not to take any action to control or otherwise limit a PMN chemical, the submitter is free to manufacture or import the chemical following expiration of the 90-day review period. Upon commencement of manufacture or import, the submitter is required to provide written notification of this fact to the EPA, after which the chemical will be placed on the inventory of existing chemicals. To date, a notice of commencement of manufacture or import has been received on approximately 50% of all PMNs submitted to the Agency (see Figure B-2).

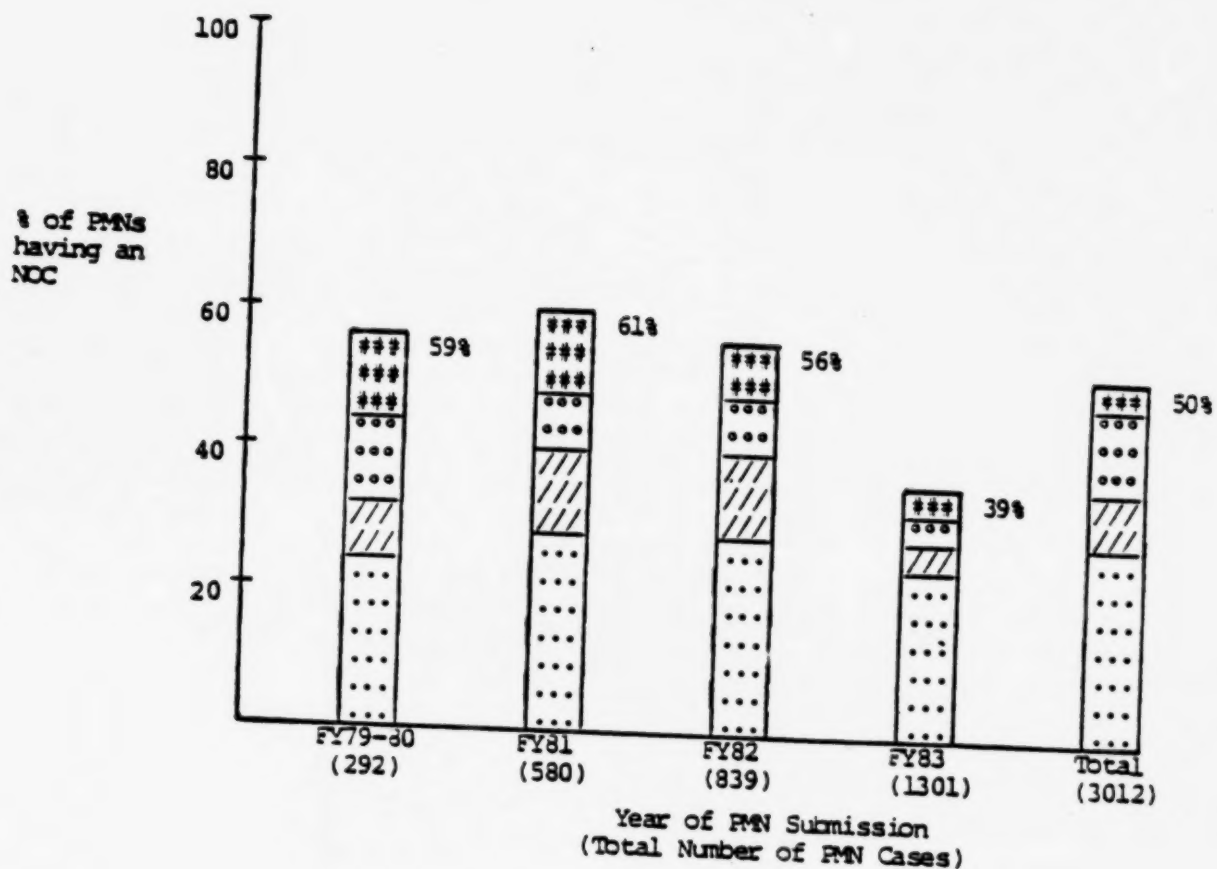
EPA's use of SAR in reaching PMN hazard assessment conclusions has been the subject of questioning and some criticism from the Congress, environmental groups, and others (Office of Technology Assessment, 1983; General Accounting Office, 1984; American Chemical Society, 1984), who point out the many uncertainties associated with the approach and question whether it is adequate for purposes of chemical regulation.

BACKGROUND

The PMN Assessment Process

Figure B-3 presents a schematic of the overall PMN risk assessment process employed by EPA. The first step in the process is a determination that all necessary information has been included in the notification. This is followed by a series of three meetings which bring senior level expertise to bear on the questions of chemistry, hazard, and exposure within the first 15 days of the 90-day period available to EPA for the assessment of each PMN chemical. Although generally characterized as "professional judgment" meetings, the initial discussions are supported by a variety of information gathering activities, including (1) identification of previously received PMN chemicals which structurally (or otherwise) resemble the newly submitted chemical, (2) substructure- and

*A "consent" 5(e) order is one in which EPA negotiates the terms of the order with the company that submitted the PMN. The company agrees to be bound by the order and waives its rights to file objections to the order. This waiver does not affect any other rights that the company may have under TSCA. The company at a later date can request a modification of the consent order. This is contrasted with a unilateral order under which EPA takes action to restrict or prohibit the manufacture or use in commerce of the PMN substance.



Legend: Days after day 90 that the Notice of Commencement is received.

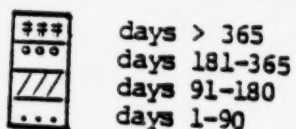
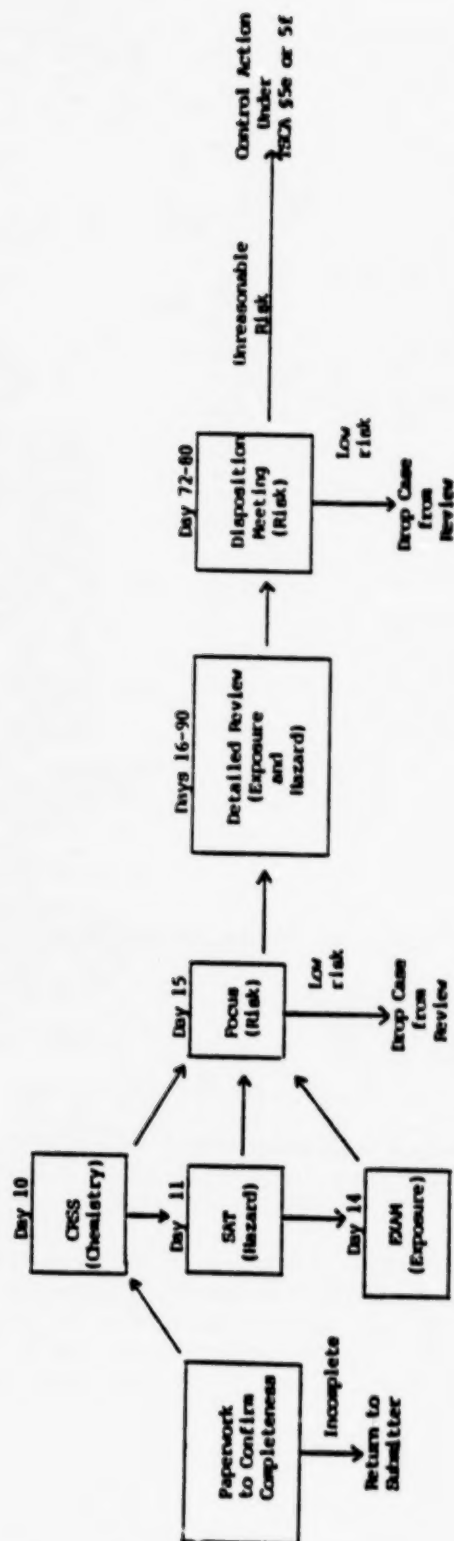


Figure B-2. Statistics on receipt of notices of commencement of manufacture or import (NOC; see explanation of an NOC in the text) for PMNs received between fiscal year 1979 and fiscal year 1983 (EPA, 1984b). FY = fiscal year.

nomenclature-based searches to identify potential analogues, (3) searches in handbooks and bibliographic data bases to identify pertinent literature on the PMN chemical or its potential analogues, (4) critical review of submitted test data, with special emphasis on toxicologic testing, and (5) calculation of a variety of physical chemical properties, including water solubility (Banerjee et al., 1980), K_{oc} , vapor pressure (Lyman et al., 1982), log P (Chou and Jurs, 1979), and bioconcentration factor (Veith et al., 1979).



Legend: CRCS - Chemistry Review and Search Strategy
 SMT - Structure Activity Team
 EXAM - Exposure Analysis Meeting

Figure B-3. PMN assessment process.

The first of these meetings, known as Chemistry Review and Search Strategy (CRSS), (1) confirms the chemical identity of the substance, in terms of matching the name with the structural depiction provided, (2) considers (or develops as needed) the PMN chemical's industrial synthetic process to identify by-products or impurities which might be present (if by-products and/or impurities are identified by submitter, the task is one of explaining and expanding on the information provided), (3) collects and/or estimates values of various physical chemical properties, and (4) attempts to understand how the substance functions chemically in its projected use applications.

The second professional judgment meeting is known as the Structure Activity Team (SAT) and is responsible for the initial assessment of the potential hazards posed by the chemical. The SAT is composed of senior scientists who specialize in organic chemistry, environmental chemistry, metabolism of xenobiotic chemicals, chemical carcinogenesis and mutagenesis, systemic toxicity, teratogenesis, and environmental toxicology. SAT consideration of a PMN chemical commences with a presentation by the CRSS chairman of the findings of that group. This is followed by discussions of (1) environmental fate, (2) health effects, and (3) environmental effects. The health effects discussion considers uptake, metabolism, distribution, excretion, and any specific toxicities suggested by the PMN chemical's structure or identified through preliminary information searches; test data provided with the notice are also discussed and evaluated at this time. The environmental effects discussion considers acute and chronic aquatic toxicity, bioconcentration potential, and terrestrial toxicity.

The final professional judgment meeting is termed the Exposure Analysis Meeting (EXAM) and is responsible for consideration of the nature and magnitude of (1) occupational exposure associated with manufacture, processing, and use of the new chemical, (2) environmental releases resulting from these activities, as well as disposal of chemical wastes, (3) consumer and environmental exposures (including drinking water), and (4) environmental fate (transport and transformations).

The three professional judgment meetings are followed by the initial risk assessment meeting, termed the Focus meeting. The Focus meeting is chaired by a regulatory decisionmaker and brings together the chairpersons from the chemistry, hazard, and exposure meetings for synoptic presentations of the conclusions from each previous meeting, followed by open discussion of the risk potential presented by the PMN chemical. Using the information and/or judgments developed to this point, a decision is reached at the Focus meeting to either drop the chemical from further consideration (approximately 60% [EPA, 1984a] of all cases) or to pursue further review of the chemical, a process known as a "Detailed Review." * Thus, at the crucial initial stages of the PMN review period, EPA has devised a "screening" procedure which relies on senior expertise to distinguish those PMN chemicals which are potentially hazardous and/or might have significant exposure and release potential from other chemicals considered unlikely to present a significant risk for injury to health or the environment.

*Another option available at Focus (as well as subsequent points in the assessment process) is to enter the chemical into the "Followup" program, which would consider the need for promulgation of a "significant new use rule" under TSCA section 5(a)(2) or an information reporting rule under section 8(a). Chemicals controlled under section 5(a)(2) are subject to the same 90-day notification requirement as for new chemicals, except that the need to report is triggered by the development of one or more significant new uses not outlined in the original premanufacture notification submitted to EPA. These new uses are then examined to determine if they "may" or "will" present an unreasonable risk under section 5. Section 8(a) reporting rules, on the other hand, only require the submission of certain information and do not trigger automatic review under section 5.

During the Detailed Review, those aspects of the case identified for further consideration at the Focus meeting are examined in depth by discipline experts. All available data on the PMN substance and its analogues are gathered and reviewed by the experts, who prepare a written evaluation of the data and make appropriate recommendations. These recommendations are then reviewed by senior scientific and managerial staff, who determine the nature and magnitude of potential hazards and then examine whether there is potentially sufficient exposure that the chemical might pose a significant risk. If the decision is reached that the chemical might pose a risk, the case is presented to senior regulatory decisionmakers at a "Deposition" meeting; they decide whether the risk is "unreasonable." The possible outcomes of this meeting include (1) to control the chemical pending completion of needed testing under TSCA section 5e, (2) to directly control the chemical under TSCA Section 5f, or (3) to drop the case from further consideration. All of the above decisions turn on the strength of the case made for an "unreasonable risk" determination with, as noted before, TSCA section 5e requiring a "may present" finding, TSCA section 5f requiring a "will present" finding, and a drop resulting from inability to meet either of these tests.

The PMN Universe

Over 4000 chemicals have been submitted to EPA under section 5 of TSCA since mid-1979. The ratio of polymeric to nonpolymeric PMN chemicals is approximately 40:60. For the set of nonpolymeric PMN chemicals, the mean molecular weight is approximately 450 daltons, and the mode is between 200 and 300 daltons; the range of molecular weights extends from 104 to over 4000 daltons (see Figure B-4). Table B-1 presents the most commonly encountered functional groups among the Detailed Review cases and also includes baseline occurrence values for the listed functional groups. Table B-2 identifies the most important functional groups among the Detailed Review cases. These functional groups were selected on the basis of (1) their appearance in $\geq 5\%$ of the Detailed Reviews and (2) a ratio of Detailed Reviews to baseline cases of ≥ 0.15 (i.e., about 1 in 6).

Test Data Received in PMNs

As noted earlier, TSCA section 5 does not require the provision of test data with incoming PMN submissions. Rather, submitters must provide any test data that are available to them at the time of submission. Table B-3 presents summary statistics for test data submitted with PMNs. As can be seen, 52% of all PMNs contain no test data of any type; the situation is somewhat better for nonpolymeric PMN chemicals, of which only 39% do not contain any test data. In general, health test data are more commonly provided than ecotoxicological or environmental fate data. Among the various types of health test data provided, the most commonly received studies are oral and dermal acute toxicity and skin and eye irritation studies, with mutagenicity, dermal sensitization, and "other" health studies appearing less frequently. "Other" health test data include repeated-dose toxicity studies, teratogenicity assays, phototoxicity studies, and a variety of other toxicity studies. (The reader is also referred to an earlier evaluation of the test data provided in PMNs prepared by the Office of Technology Assessment, 1983.)

Table B-4 summarizes the combinations of different health test data most commonly encountered among PMNs containing health data. Thus, this table gives an indication of the extent of the testing that is undertaken for those cases that contain health test data. The most frequently encountered combinations of tests submitted with PMNs consist of acute toxicity (any route) and local irritation studies (34% of the 1560 PMN cases containing health test data); for the most part, these take the form of the battery required for labeling purposes under the Federal Hazardous Substances Act (U.S. Code, Title 16, Section 1500). An equal number (522 or 33%) of the health-data-containing cases include acute toxicity (any route) and irritation studies (both skin and eye) in combination with any one or more of sensitization, mutagenicity, or "other" health test data. Sixty (or 4%) of the data-containing cases contain only sensitization, mutagenicity, and/or "other" health test data (thus, they do not contain acute or local toxicity testing). Table B-5 reports the number of

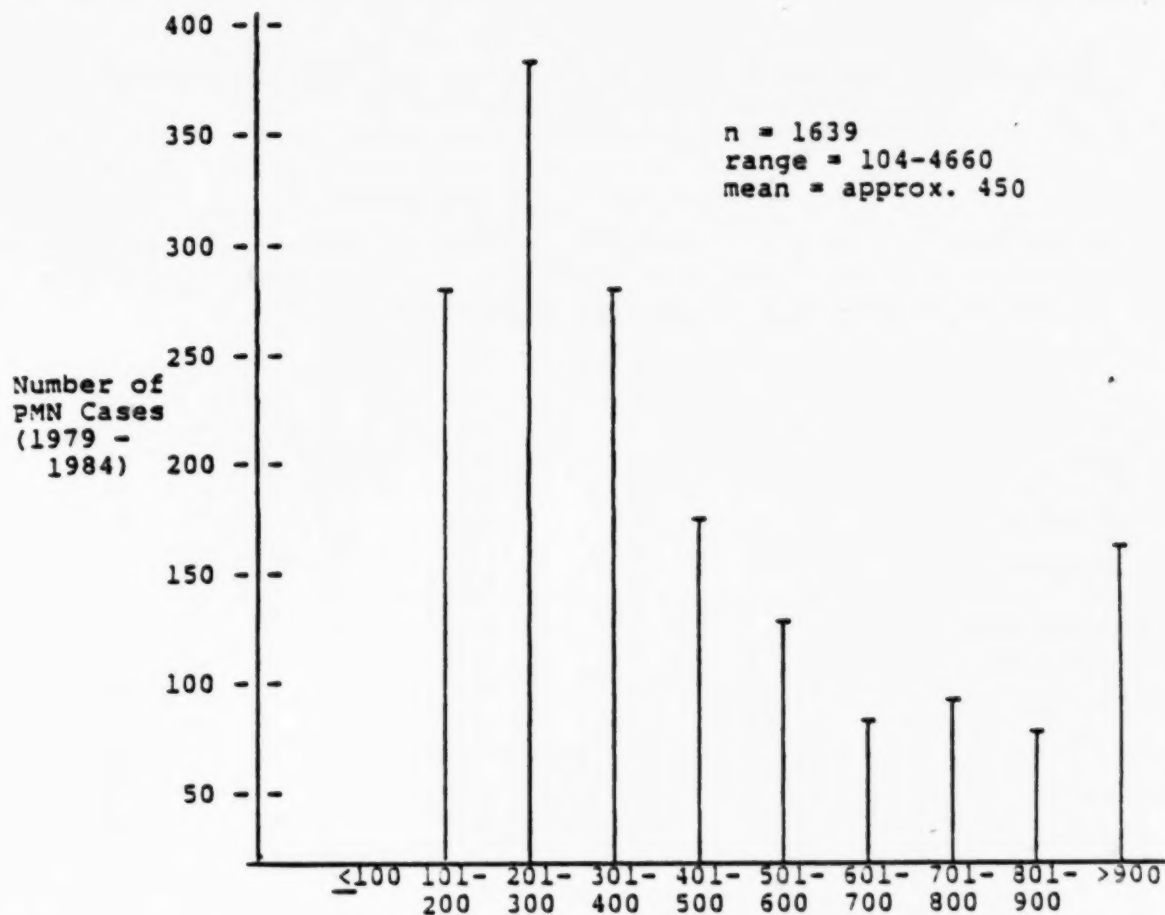


Figure B-4. Molecular weight distribution (in daltons) for nonpolymeric chemicals (EPA, 1984b).

TABLE B-1. FUNCTIONAL GROUP OCCURRENCE AMONG ALL PMNs (BASELINE) AND THOSE ENTERING DETAILED REVIEW (1979 - FY 83)

Functional Group	Detailed Review Cases		Baseline PMNs		% Baseline Entering Detailed Review
	Number	Percent	Number	Percent	
A. <u>All PMN Chemicals (320 Detailed Review Cases; 3099 Baseline PMNs)</u>					
Amines	102	32	817	26	12
Azo	73	23	254	8	29
Ether	55	17	791	26	7
Phenol	54	17	352	11	15
Sulfonic Acid	53	17	337	11	16
Chlorocarbon	52	16	248	8	21
Ester	35	11	1189	38	3
Aliphatic OH	33	10	705	23	5
Amide	30	9	354	11	8
Long Chain Alkyl	27	8	629	20	4
Carboxylic Acid	23	7	535	17	4
Alkene	22	7	573	19	4
Fluorocarbon	22	7	130	4	17
Nitro	19	6	106	3	18
Sulfate	18	6	34	1	53
Sulfide	17	5	135	4	13
Ketone	15	5	154	5	10
Sulfone	15	5	61	2	25
B. <u>Nonpolymeric PMN Chemicals (289 Detailed Review Cases; 1796 Baseline PMNs)</u>					
Amines	93	32	624	35	15
Azo	71	25	246	14	29
Phenols	51	18	278	16	18
Sulfonic Acid	51	18	300	17	17
Chlorocarbon	50	17	213	12	23
Ether	49	17	350	20	14
Amide	23	8	171	10	13
Aliphatic OH	22	8	247	14	9
Long Chain Alkyl	21	7	333	19	6
Ester	21	7	350	20	6
Fluorocarbon	21	7	96	5	22
Alkene	19	7	273	15	7
Nitro	19	7	106	6	18
Carboxylic Acid	17	6	204	11	8
Sulfide	15	5	107	6	14
Ketone	15	5	132	7	11
Sulfone	15	5	52	3	29
Sulfate	15	5	30	2	50
Imine	14	5	100	6	14

(continued)

TABLE B-1. Continued

Functional Group	Detailed Review Cases		Baseline PMNs		% Baseline Entering Detailed Review
	Number	Percent	Number	Percent	
C. <u>Polymeric PMN Chemicals (31 Detailed Review Cases; 1303 Baseline PMNs)</u>					
Ester	14	45	839	64	2
Aliphatic OH	11	35	458	35	2
Amine	9	29	193	15	5
Amide	7	23	183	14	4
Ether	6	19	441	34	1
Long Chain Alkyl	6	19	296	23	2
Carboxylic Acid	6	19	331	25	2
Quaternary N	5	16	34	3	15
Alkene	3	10	300	23	1
Phenol	3	10	74	6	4
Carbamate	3	10	205	16	1
Sulfate	3	10	4	0.3	75

PMNs which contain one category of health test data, two categories of test data, and so on through the eight health test data categories included in TDIS, an EPA in-house PMN information system.

The amount and types of health test data provided with PMNs can be contrasted with the data estimated (National Research Council, 1984) to be available on chemical substances contained in the TSCA inventory of existing chemicals. Relatively more data are available to the set of PMN chemicals than is the case for TSCA existing chemicals. However, in absolute terms, available data on PMN chemicals for end points other than acute toxicity and local irritation are limited. This is a serious limitation, since studies such as sensitization, mutagenicity, reproductive/developmental toxicity, repeated dose toxicity, and so on, are generally viewed as being more critical to an overall assessment of health hazards than are acute studies.

The Use of SAR in PMN Health Hazard Assessments

Given the limitations of the test data provided with PMNs, EPA has evolved a reliance on structure-activity relationships in its evaluation of the potential hazards posed by PMN chemicals. EPA's operational definition of "structure-activity relationships" was described above as being comprised of four components. The first of these components, submitted test data, has already been discussed. The second of the components, data on analogous substances, deserves some comment. In order for an analogue to be useful to EPA, it must resemble the PMN chemical in one or more critical aspects (e.g., structurally, substructurally, physicochemically, etc.) and at the same time must have pertinent toxicologic data available on it in the scientific literature. It has been EPA's experience, and the point was recently confirmed by the National Research Council (1984), that available test data are very limited for most chemicals, but especially so for TSCA chemicals. This factor becomes a major limitation on the usefulness of many potential analogues.

TABLE B-2. "MOST IMPORTANT" FUNCTIONAL GROUPS AMONG THE DETAILED REVIEW CASES

Functional Group	% of All Detailed Reviews	Ratio of Detailed Review/Baseline	Percent
<u>All PMNs</u>			
Azo	23	73/254	29
Phenol	17	54/352	15
Sulfonic Acid	17	53/337	16
Chlorocarbon	16	52/248	21
Fluorocarbon	7	22/130	17
Nitro	6	19/106	18
Sulfate	6	18/ 34	53
Sulfone	5	15/ 61	25
<u>Nonpolymeric PMNs</u>			
Amines	32	93/624	15
Azo	25	71/246	29
Phenol	18	51/278	18
Sulfonic Acid	18	51/300	17
Chlorocarbon	17	50/213	23
Fluorocarbon	7	21/ 96	22
Nitro	7	19/106	18
Sulfone	5	15/ 52	29
Sulfate	5	15/ 30	50
<u>Polymeric PMNs</u>			
Quarternary N	16	5/ 34	15
Sulfate	10	3/ 4	75

Analogous substances may be identified by either EPA or the submitter. The latter instance most often takes the form of test data on structurally related substances which are existing chemicals produced by the submitter. The data provided tend to resemble in scope the types described earlier as typically accompanying PMN submission (i.e., acute toxicity and local irritation studies, occasionally mutagenicity or repeated dose toxicity studies). EPA relies on two sources in its internal effort to identify chemical analogues. These consist of analogue recommendations offered by members of the Structure Activity Team and other technical staff and structural analogues retrieved from several publicly available automated chemical substructure and nomenclature search systems.*

*Examples of available substructure and nomenclature search systems include SANSS (Structure and Nomenclature Search System) in the NIH/EPA Chemical Information System, CAS-ONLINE available from Chemical Abstracts Service, and DARC, a French system available from Questel.

TABLE B-3. TEST DATA SUBMITTED WITH PREMANUFACTURE NOTICES^a

Type of Data	Percent of PMNs		
	All	Non-Polymer	Polymer
Health data (some)	46	58	30
Acute Toxicity			
Oral	39	51	24
Dermal	21	26	14
Inhalation	8	10	7
Local Toxicity			
Eye irritation	34	45	22
Dermal irritation	37	48	23
Sensitization	9	12	5
Mutagenicity	13	19	5
Other ^b	8	12	4
Ecotoxicological data (some)	10	14	6
Acute lethal vertebrate	7	11	4
Acute lethal invertebrate	3	4	2
Fate data (some)	10	12	5
Biodegradation	6	9	3
Log P	3	6	1
No test data of any type	52	39	68

^aBased on the full set of 3,578 PMNs received to date (6/84) (EPA, 1984b).

^bThe "other" health category includes studies of acute toxicity by other routes (ip, iv, etc.), repeated-dose toxicity (generally 28 days or less in duration), teratogenicity, phototoxicity, neurotoxicity, and a variety of other end points.

In the former instance, the proposed analogues often provide a rich source of pertinent information which can be applied to the assessment effort. The Structure Activity Team and other technical staff also provide guidance in constructing the strategy for the automated analogue searches. This guidance consists of the identification, based on experience and professional judgment, of the putative toxicophore(s) within the PMN chemical's structure. Potential analogues resembling the PMN chemical in the structure or function of the putative toxicophore(s)* are then identified via an

*A "functionally similar" chemical is one which, although it differs substructurally, can be considered a functional equivalent of the PMN chemical. Examples of functional equivalents, depending on the specifics of the case, might include acceptance of an aromatic amine substituent in lieu of an aromatic nitro (based on the expectation of biotransformation) or a chloro (but generally not a fluoro) in lieu of a bromo substituent (based on the concepts of isosterism; see Burger, 1970, and Mathison et al., 1976).

TABLE B-4. MOST FREQUENTLY ENCOUNTERED COMBINATIONS OF HEALTH TEST DATA
SUBMITTED WITH PMNs^{a,b}

Number of PMNs	Acute Toxicity			Irritation		SENS	MUTA	OTHR
	ORL	DRM	IHL	DRM	EYE			
256	X			X	X			
177	X	X		X	X			
121 ^c	X	X	X	X	X	X	X	X
100	X	X	X	X	X			
72	X							
70	X	X		X	X		X	
63	X			X	X		X	
50	X			X	X	X		
34				X	X			
33							X	
31	X	X		X				
28	X	X	X	X	X		X	
28	X			X	X	X	X	
28	X			X				
26	X			X	X		X	
25	X	X		X	X	X		X
24	X			X	X	X		
21	X			X	X		X	X
20	X	X		X	X	X	X	
19	X	X		X	X			X
19	X		X	X	X			
18	X	X		X	X	X	X	X
17								X

^aBased on 1646 PMN cases containing some type of health test data (8/84), (EPA, 1984b); approximately 400 cases contain 94 other combinations of health test data.

^bAbbreviations as follows: ORL = oral; DRM = dermal; IHL = inhalation; EYE = eye; SENS = sensitization; MUTA = mutagenicity; OTHR = other health studies.

^cIncludes 106 synfuels received as a group.

automated searching capability. In selection of analogues for literature searching and subsequent assessment purposes, similarity in the structure or function of the toxicophore is an essential element. Physical chemical properties, especially those that are known or suspected of contributing to a chemical's biological activity, as well as other major aspects such as projected metabolic pathways for the potential analogues as compared with those of the PMN chemical, are also considered in selecting potential analogues.

One of the major limitations of the available substructure and nomenclature search systems is that they restrict the searchable parameters to chemical names, name fragments, substructural

TABLE B-5. THE NUMBER OF PMNs CONTAINING 1 THROUGH 8 DIFFERENT CATEGORIES OF TEST DATA^a

Number of Test Data Categories Submitted ^b	Number of PMNs
1	151
2	109
3	383
4	370
5	333
6	126
7	53
8	121 ^c

^aBased on 1646 PMN cases containing some type of health test data (8/84), (EPA, 1984b).

^bTest data categories include acute lethal oral, acute lethal dermal, acute lethal inhalation, eye irritation, local skin irritation, sensitization, mutagenicity, and other. This table does not reflect the possible submission of multiple tests in a single category (for example, a notice containing a single mutagenicity study is not distinguished from other notices containing more than one such study).

^cIncludes 106 synfuels received as a group.

components, molecular formulae, and so on. Sizeable data bases of physical chemical properties are not available in a readily searchable environment, although several are under development (Eakin and Johnson, 1981; Milne and Heller, 1980; Magnuson et al., 1981; Howard et al., 1982; Page, 1983; Page and Kissman, 1984). Thus, automated screening of the analogue search outputs using quantitative measures of physical chemical properties to select potential analogues is not presently available. Consequently, EPA relies on manual screening using a variety of quantitatively and/or qualitatively applied factors for comparison between the potential analogues and the PMN chemical. These factors can include relative differences in, for example, molecular weight, molecular topology, log P (Hansch and Leo, 1979), presence and positioning of reactive or potentially reactive groups, possible steric effects, presence of aromatic systems, and presence of ionizable (Perrin, 1965; Perrin, 1972; Serjeant and Dempsey, 1979) or zwitterionic groups. Once potential analogues have been selected, they are subjected to automated literature searching using a variety of readily available bibliographic systems and data bases in the hopes of uncovering pertinent toxicity information. Only those analogues yielding test data are carried forward in the assessment process.

The use of the third component, quantitative SAR methods, is at present limited to the estimation of certain physical chemical properties, such as water solubility (Banerjee et al., 1980), K_{oc} , vapor pressure (Lyman et al., 1982), log P (Chou and Jurs, 1979) and ecotoxicity (prediction of acute LC_{50} s in aquatic organisms (Veith et al., 1983; Lipnick and Dunn, 1983; Hermans, 1983; Konemann, 1981; Hutzinger et al., 1978) and estimation of bioconcentration factors (Veith et al., 1979).

The last of the four components, the knowledge and professional judgments of scientific assessors in the interpretation and integration of available information, is most critical in terms of the overall success of the evaluation effort. Given that the three preceding components, even when combined, will generally produce a limited set of useful information, the importance of the knowledge and professional judgments of the scientific assessors becomes apparent. This is especially so in the case of information developed on analogous substances which must be critically evaluated and interpreted in terms of the weight that should be applied to each analogue as a

function of its "closeness" to the PMN chemical. Thus, the assessors' task is to evaluate the toxicologic potentialities of the PMN chemical using submitted data and extrapolations of data available on suitable analogues. In performing this task the assessors must consider a variety of parameters as they apply to the PMN chemical and, in comparison, to the analogue chemicals. These parameters include those applied previously in selecting potential analogues, as well as

- potential for skin, pulmonary, and gastrointestinal absorption;
- biotransformation pathways;
- distribution and excretion;
- consideration of the possible mechanisms of toxicity; and other parameters (Arcos, 1983; Arcos and Auer, in press).

In making projections about the toxicity of the PMN chemical, EPA is hampered by the limited data that are generally available on the PMN chemical and its analogues. Because of this, EPA's hazard predictions tend to take the form of conservative, worst-case analyses which reflect the uncertainties inherent in a process which uses limited test data. The TSCA section 5e "may present" language recognizes the uncertainties that are likely to confront EPA in assessing PMN chemicals and thus allows a less robust regulatory finding to suffice in requiring the development of the test data needed to adequately assess the risk posed by new chemicals.

GENERAL QUESTIONS FOR CONSIDERATION AT THE WORKSHOP

(1) Given the data limitations that EPA must operate under in assessing the potential hazards of PMN chemicals, which of the available QSAR and other computational approaches might offer the best potential to provide a rational, scientific basis for PMN hazard decisions (a) over the short term and (b) over the longer term? What are their strengths and weaknesses?

(2) EPA's present approach to PMN hazard assessment relies heavily on efforts to identify potential analogues of PMN chemicals which (a) structurally or otherwise resemble the PMN chemical and (b) have biological test data available on them in the scientific literature. What techniques or combinations of techniques might be useful in measuring the degree of similarity between potential analogues and the PMN chemical? These techniques can operate at two levels, in the first instance to screen possible analogues and thus obtain a reasonably sized set of candidates and in the second instance to quantify chemical similarity between the PMN chemical and its potential analogues. Which of these techniques might be useful over the short term? Which should be pursued as part of a research effort to improve the existing capabilities?

(3) Prediction of biotransformation pathways and metabolites is an important aspect in PMN hazard assessment. What techniques or approaches (short- or long-term) might be most useful to EPA in improving its capabilities in this area?

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APPENDIX C

PREDICTIVE BIOLOGY FOR COMPLEX ENVIRONMENTAL SITUATIONS AND STUDIES

Larry D. Claxton

Obviously, in environmental situations, individuals are normally exposed not to a single environmental pollutant, but to a vast array of pollutants. For example, air pollution obviously can exist in almost any defined environment. Cigarette smoking contributes to pollutant levels in homes, businesses, and occupational settings. Automotive exhaust has some measurable impact upon most outdoor areas. Fugitive emissions are associated with many industrial processes. Each of these sources emits many different toxic pollutants. In addition, most defined environments contain pollutants from a mixture of sources, and these sources contribute proportionately differing amounts of organic and inorganic materials. To complicate the picture even further, these atmospheric pollutants interact in various ways depending upon the environmental conditions present (e.g., temperature, light, humidity, etc.). Individuals, therefore, are exposed to a very large number of organic and inorganic pollutants, each of which is present in relatively minute quantities and many of which are secondary rather than primary pollutants. In spite of these generally accepted observations, most health research has examined the toxicology of source compounds, which include (1) end-products or major by-products of an industrial process, such as trichloroethylene, (2) compounds that are readily identified just prior to and immediately after release into the atmosphere, such as benzo(a)pyrene associated with coke oven emissions, and (3) compounds that are in common use within the environment and are likely to exhibit some form of toxicity, such as pesticides. Since these source compounds are subject to chemical reactions within the atmosphere, most populations of interest may not be exposed to these source compounds. Instead, they may be exposed to secondary pollutants (i.e., reaction by-products) of these compounds. (Occupational populations are likely to be an exception.) Although the testing of source chemicals is the initial logical outcome of trying to identify and determine the toxicity of environmental pollutants, present efforts are beginning to concentrate upon the identification and toxicological testing of pollutants to which the population is actually exposed.

Although there are a variety of possible uses for predictive biological techniques for these complex mixtures, I would like to point out only three for consideration.

- (1) Establishing the priority for the testing of newly identified environmental chemicals.

During recent years, in an effort to identify the airborne organic toxicants to which the population at large is exposed, a variety of ambient studies have been conducted. In some of these studies, respirable particles have been collected and extracted. The extracted organics have then been subjected to bioassay-directed fractionation procedures in order to identify genotoxic fractions and/or chemicals. These qualitative procedures provide lists of identified and "tentatively identified" compounds. As these procedures improve, more and more compounds for which there are no or inadequate toxicological data are identified. The first need, therefore, is to establish a priority for the toxicological testing of these newly identified compounds. This situation is analogous to the PMN situation.

- (2) Predicting which compounds have high probability of being in chemical classes that are refractory to chemical analysis.

When environmental samples are fractionated as described above, some of the organic fractions that are mutagenic are refractory to further analysis for the identification of specific

mutagens. For example, the very polar fractions associated with diesel emissions are very mutagenic in bacterial bioassays; however, presently available procedures have not been able to identify the mutagenic components. If investigators knew which compounds within these fractions were likely to account for the mutagenicity of the fraction, then procedures for the actual identification and testing of these fractions and compounds could be expedited.

- (3) Predicting the trace organic components produced by atmospheric reactions.

In order to bridge the knowledge gap between source compounds known to be emitted into the atmosphere and compounds found in ambient air samples, EPA has been involved in a number of atmospheric transformation studies that involve the monitoring of genotoxic activity. Early in these studies, we found that irradiated mixtures of propylene, NO_x , and SO_2 produced mutagenic products. Although this well-known smog reaction was thought to be "extremely well characterized," the known products of reaction do not account for the mutagenicity observed. Since many of the reactions are understood and since the active constituents are volatile and do not condense within 24 hours, can one go beyond present methods and postulate what the trace but highly active components are?

Questions to Consider

- (1) What SAR methods are most appropriate for establishing the priority for the toxicological testing of newly identified environmental chemicals? An additional component to this question is, can one also establish a priority listing of the bioassays needed for each of the selected compounds?
- (2) When chemical fractionation is used, can one predict component compounds in refractory fractions?
- (3) Can one establish better methods for predicting the trace mutagenic components resulting from atmospheric transformation processes?

APPENDIX D

CARCINOGENESIS AND MUTAGENESIS (GENETIC TOXICOLOGY)

BACKGROUND

The application of structure-activity techniques to the field of carcinogenesis has received much attention in the last 30 years. The pioneering work of the Daudels, Pullmans, and other coworkers on the relationship of theoretical calculations based on electronic structure to the carcinogenic activity of polycyclic aromatic hydrocarbons (PAH) has led to current theories of bay-region activation and the importance of $\Delta E_{\text{deloc}}/\beta$ as described by Jerina et al. (1976). These theories gain support from confirming studies on the metabolism and metabolic activation of PAH and from DNA-adduct analyses. Additional structure-activity relationships have been developed with PAH using other indices and methods [e.g., Hansch multiple linear regression and pattern-recognition]. Chemical carcinogens of other chemical classes have also received attention in terms of SAR analyses, notably, aromatic amines and nitrosamines. However, many classes of chemical carcinogens, including the following, have not received much attention with respect to SAR analyses: metals, hydrazines, hormones, halogenated organics, azo dyes, epoxides, aziridines, and nitroaromatics.

The carcinogenesis process is complex because it encompasses many phenomena: metabolism and related pharmacokinetic and pharmacodynamic processes; DNA binding and repair; tumor promotion and tumor progression; and cell, tissue, organ, strain, sex, and species specificity. Most structure-activity studies which relate chemical structure to carcinogenic activity are based on simple molecular properties which do not seem to be related to the phenomena involved in chemical carcinogenesis.

The field of chemical mutagenesis, or, more broadly, genetic toxicology, has only recently been a subject of structure-activity relationship studies. Several reports correlating results in *Salmonella typhimurium* bacterial gene mutation studies with chemical structure have been published. Gene mutation is a much "simpler" end point than carcinogenesis, often a result of one base-pair change. The activity of direct-acting carcinogens or mutagens (i.e., those not requiring metabolic activation for activity) in bacterial or mammalian cell gene mutation bioassay systems would seem to be a good subject for SAR studies. Other genetic toxicology end points which could be studied are chromosomal aberrations, DNA damage and repair, and oncogenic cell transformation. However, except for DNA damage and repair, these end points probably represent the effects of multi-hit kinetics and are the result of complex events.

MAJOR ISSUES

- (1) Many carcinogens or mutagens are not active in their native form and require metabolic transformation to active, often electrophilic, intermediates. What approaches or techniques can be employed to incorporate these transformations in SAR studies?
- (2) The solubility, uptake, distribution, and transport of chemicals in biological test systems need to be factored into SAR studies. How can this be achieved?
- (3) When utilizing biological effects that are the result of complex phenomena, how can the SAR analyst take into account as much as is known of the mechanisms of action of the chemicals or processes studied?

(4) Many SAR studies use homogeneous data sets. What methods can be applied to study heterogeneous data sets?

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APPENDIX E

TERATOLOGY

The science of teratology concerns all aspects of the production of birth defects, the causes, mechanisms, and precise sequelae of perturbation of the normal developmental processes. These processes are an integrated continuum of genetic, biochemical, biophysical, and morphogenic factors. Alterations in any of these factors may be manifested in the offspring as deviations of a morphological and/or functional nature. It is estimated that 3% of fetuses surviving to birth have recognizable defects, and in an additional 3 to 4%, defects become recognizable later in life. It has been estimated that 10 to 15% of the identified birth defects have a genetic etiology, approximately 15% are due to intrauterine infections, 2 to 3% can be considered to be due to environmental factors, and the remainder are the resultant of a complex and unknown etiology which may encompass any of the above factors.

To date, fewer than 25 agents have been proven to affect human development. Their nature is diverse, and they include the following:

- Environmental agents: methyl mercury
- Drugs: androgenic hormones, aminopterin, diethylstilbesterol, thalidomide, alcohol, valproic acid
- Infections: Rubella, cytomegalovirus
- Physical factors: radiation, hypoxia

The effects that have been confirmed include morphological birth defects, predisposition to cancer, intrauterine growth retardation, and central nervous system deficits that may result in impaired motor function and/or intelligence.

It is recognized that although the number of confirmed human teratogens is small, there are hundreds of agents that have been shown to produce birth defects in laboratory animals and thousands more for which we have no data on teratogenic potential. There is, therefore, great difficulty in assessing the potential human risk of environmental agents. To assess risk, it is essential to focus both on the developmental processes which are the targets for toxicity and on the chemicals being examined. Along with adaptive, homeostatic, and repair processes of the biological organism, the pharmacodynamic and pharmacokinetic factors must be carefully considered. Pharmacodynamic mechanisms describe the molecular interactions which determine dysfunction; pharmacokinetic factors govern the amount and form of the chemical which reaches its receptor for toxicity.

In the area of teratology, we still have relatively little information on agent-specific mechanisms of action. The critical regulatory problems are, therefore, twofold. The first involves the extrapolation of data derived from animal bioassays to the human population. While regulatory agencies have made decisions based upon teratology test data, and will continue to do so, a number of examples of the difficulties inherent in this process are always evident. In the case of thalidomide, a sedative which produced thousands of serious human malformations in the late 1950's and early 1960's, the commonly used laboratory species of rodents did not react to its administration during pregnancy. Numerous hypotheses have been advanced to explain its teratogenic activity and

interspecies differences, but to date there has been no clear elucidation of either a mechanism(s) or a structure-activity relationship(s) which would clearly explain the vast array of data collected by teratologists. The salicylates (aspirin) provide the reverse situation. Although there is no evidence of any teratogenic effects in humans taking large quantities of this drug, it remains the classic teratogen in rats, as it produces a variety of serious defects in this species at relatively low doses. Finally, in the case of vitamin A and its congeners, teratogenic potential in numerous animal species is well known, but a safe level for human intake could not be definitively determined, and a drug preparation containing retinoic acid has recently been implicated in the formation of human birth defects.

The second critical regulatory problem concerns the setting of priorities for testing the vast array of unknown agents by standard teratology bioassays. The present lack of information on the basic mechanisms makes it difficult to set these priorities on a causal basis. There are examples of groups of similar compounds that have been shown to have widely divergent teratogenic potential, including thalidomide congeners and the biphenyl ethers. Series of compounds in both of these groups have been tested in a variety of species, and results have varied from extreme teratogenic response to no effects.

It is obvious that teratology must move from a taxonomic phase to intensive study of basic mechanisms. It is to be hoped that data assays of animal responses to classes of compounds can enable biophysicists and biochemists to hypothesize potential mechanisms which can then be appropriately tested.

APPENDIX F
DIOXIN CASE STUDY
WORKSHOP ON COMPUTATIONAL TECHNIQUES IN BIOLOGICAL PROBLEMS

R.A. Stanley

BACKGROUND

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is one of the most toxic chemicals known. This is one of the 22 distinct tetrachlorinated isomers. Seven other homologous groups, with one to eight chlorine atoms, contain 53 other chlorinated dibenzo-p-dioxins.

The extreme toxicity of 2,3,7,8-TCDD is thought to be related to its molecular structure, a chlorine-substituted, planar multi-ring chemical of roughly rectangular shape, about $30 \text{ \AA}^2$. Molecules with maximum toxicity have chlorine atoms at each of the corners of the rectangle and must be absent from at least one hydrogen atom at the other possible points of substitution.

A number of chemicals have a chemical structure resembling the substitution pattern and share biological activity to some degree. Chlorinated dibenzofurans, with 135 congeners, are notable among them. In addition, some brominated dioxins and furans; polychlorinated and polybrominated biphenyls; and halogenated biphenylenes, azoxybenzenes, and naphthalenes are structurally related to chlorinated dioxins. In all, about a thousand chemicals bear some degree of relationship.

Many of these halogenated multi-ring chemicals exhibit strong binding to a receptor protein (called the Ah receptor), accumulation of the Ah receptor in the nucleus, and induction of monooxygenases of aromatic hydrocarbons (AHH). It is not clear whether these effects are related to the mechanism of action of dioxins, but correlations exist between the structural characteristics of chlorinated dioxins (as outlined above) and their relative potency for Ah binding, AHH induction, and various measures of *in vivo* toxicity. Some of the known correlations are given in Table F-1.

The Agency needs information on relative toxicity, as well as environmental releases, to assess the health risks of these compounds in a scientifically defensible manner. Various chemical processes and incineration conditions produce chemicals of these types in different amounts. Ideally, we need to know the amounts of each chemical and its relative toxicity to be able to predict toxicity of any of a number of mixtures. Potentially, a biological assay of toxicity of the mixtures themselves would also serve as data for a risk assessment. Otherwise, we will not be able to take appropriate regulatory action. The Agency is currently proposing to list certain other wastes as acute hazardous wastes based on occurrence of tetra-, penta-, and hexa-chlorinated dibenzo-p-dioxin and dibenzo furans.

With a thousand potentially relevant chemicals, collection of the needed information needs to be done intelligently. We obviously cannot require \$2,000,000 toxicity tests on each of those chemicals. Ah binding and AHH induction are much less expensive per test than direct toxicity testing, but we still should not blindly plan to run tests on all thousand chemicals. Therefore, we need to create a research strategy, based on structure-activity relationships, known toxicities, and relative production amounts, that will tell us what other information to collect and how to collect it.

TABLE F-1. POTENCIES OF DIOXINS AND DIOXIN-LIKE MATERIALS RELATIVE TO 2,3,7,8-TCDD*

Chemical	Guinea Pig LD ₅₀	Carcino-genicity	Reprod. Effects	Receptor Binding	Enzyme Induction		
					AHH	EROD	Cell Kerat'n
<u>CDDs:</u>							
Mono through tri	< 10 ^{-4e}	--	--	<0.001-0.01e	<0.001f	--	--
2378-TCDD	1 ^a	1 ^b	1 ^c	1 ^e	1 ^e	1 ^g	1 ^g
TCDDs	<0.001 ^a	--	--	<0.01-0.16e	<0.001-0.03g	--	<0.001-0.01e
2378-PeCDD	0.67 ^a	--	--	1 ^e	0.1g	--	0.5e
PeCDDs	0.002 ^a	--	--	--	<0.001g	--	--
2378-HxCDDs	0.03 ^a	0.04 ^b	0.01 ^c	0.05e	0.7g	--	0.005e
HxCDDs	--	--	--	--	<0.001g	--	--
2378-HpCDDs	0.004 ^a	--	--	--	0.4g/0.002f	--	--
HpCDDs	0.002 ^a	--	--	--	0.002f	--	--
OCDD	--	--	--	--	0.004-0.03f	--	--
<u>CDFs:</u>							
Mono through tri	--	--	--	≤0.01d	<0.001d	<0.001d	--
2378-TCDF	0.28(0.5) ^a	--	0.03-0.1c	0.25d/0.3e	0.01-0.04d,f,h	0.09d	0.05e
TCDFs	--	--	--	0.001-0.05d,e	≤0.001d	≤0.005d	--
2378-PeCDF	--	--	--	0.13,0.7d,e	<0.3d	0.06d	--
PeCDFs	--	--	--	0.001-0.1d,e	≤0.002d	≤0.005d	--
2378-HxCDFs	0.017 ^a	--	--	0.04-0.2d,e	≤0.2d	<0.5d	--
HxCDFs	--	--	--	0.001d,e	0.002d	0.006	--
2378-HpCDFs	--	--	--	--	--	--	--
HpCDFs	--	--	--	--	--	--	--
OCDF	--	--	--	--	--	--	--

*References are indicated by superscript letters.

CASE STUDY OBJECTIVE

This is a case study that illustrates a specific ongoing problem the Agency faces. It has both short-term and long-term aspects. This problem is better defined and understood than many problems of current concern and can serve as a model to demonstrate how a research strategy can be structured using the computational techniques of biological chemistry.

MAJOR ISSUES

Can structure-activity relationships be used to predict the relative probability of production of various dioxin-like chemicals from different chemical processes? Under various conditions of incineration?

Can computational techniques be used to guide a testing strategy on the biological effects of chemicals related to 2,3,7,8-TCDD? Relative to the order of chemicals to be tested? Relative to the bioassay systems to be used?

Are there scientifically reasonable ways to make regulatory decisions while accumulating the relevant bioassay data?

How can computational techniques be used to answer critical questions and maximize the utility of further bioassay investment?

What are the relationships among toxicity tests and other types of testing (e.g., Ah binding and AHH induction)? What uncertainties are associated with each of the testing approaches?

What are the relationships between chlorinated compounds and chemicals substituted with other halogens (Br, I)? How much testing is necessary to achieve reasonable certainty about the effects of different halogens?

What can we predict from structure-activity relationships about relative toxicity and relative production amounts of the various types of chemicals related to 2,3,7,8-TCDD (i.e., dioxins, furans, PCBs)?

What is the appropriate relationship between computational techniques and direct testing?

How would our choices vary with different budget levels (e.g., at \$200k, at \$2,000k or at \$20,000k)?

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GENERAL REFERENCE

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APPENDIX G

STRUCTURE-ACTIVITY RELATIONSHIPS AND NEUROTOXIC POTENTIAL OF ORGANOPHOSPHATE ESTERS

Recognition of the mutagenic and non-biodegradable properties of chlorinated hydrocarbons has led in recent years to emphasizing the development and use of biodegradable and relatively selective pesticides. Organophosphorous (OP) esters, recognized as early as the 1930s for their pesticide qualities, are now being produced in the U.S. by the millions of pounds annually, with at least 85 different experimental and commercial OP insecticides currently in use.

Organophosphates are lipid-soluble compounds and as such are readily absorbed through intact skin, conjunctiva, respiratory tract, and gastrointestinal tract. In animals, OP esters produce an acute cholinergic effect by inhibiting acetylcholinesterase (AChE) activity through phosphorylation of the enzyme active site. Even when the acute effects of OP exposure are treated successfully, certain species will develop, after a time lag of 8 to 30 days, clinical signs of hindlimb weakness and ataxia, which may progress to paralysis. This organophosphate-induced delayed neuropathy (OPIDN) is usually classified as a mixed (i.e., sensory-motor) polyneuropathy consisting of focal lesions with distal axonal degeneration followed by secondary demyelination. The large, long fibers of both the peripheral and central nervous system appear to be selectively affected in this neuropathy, with recovery possible only in the affected peripheral nervous system tracts. Reports of humans with OPIDN cite various exposure conditions ranging from acute administration (e.g., suicide attempts) to chronic exposure (e.g., suburban gardening, large crop spraying operations, pesticide research).

Traditionally, a neuropathic OP compound (i.e., one which can produce OPIDN) has been defined as one which produces ataxia in hens. Using this criterion, the delayed neuropathic potential of many of the OP compounds has been extensively documented (Abou-Donia, 1981; Eto, 1974; Johnson, 1974, 1975). In addition to the hen test, it has been proposed that the delayed neuropathic potential of an OP compound may also be monitored biochemically by determining the ability of an acute dosage of the compound to inhibit neurotoxic esterase (NTE, an enzyme distinct from AChE) in the brain or spinal cord of the test animal. In some of the rankings listed in Johnson's review (1975), he also presents data on the degree of NTE inhibition produced by the compound, in addition to whether the compound produced OPIDN. There is a very significant positive correlation between the compounds that produce OPIDN and the compounds that inhibit NTE $\geq 70\%$ within 48 hours of dosing.

The reader will notice the preceding paragraph only labels OP compounds that inhibit NTE more than 70% as "potential" producers of OPIDN. OP compounds that do not inhibit NTE do not induce OPIDN, but not all compounds that inhibit NTE induce OPIDN. The compounds that inhibit NTE fall into two categories: (A) potentially neuropathic, such as phosphates, phosphonates, and phosphoramidates, and (B) non-neuropathic, such as phosphinates, carbamates, and sulfonates. In fact, prior dosing with the category B compounds protects the animal from the delayed neuropathic effects of the category A compounds.

What makes the A compounds potentially neuropathic, while the B compounds are protective, even though both categories may inhibit NTE? The answer is postulated to reside in the compound's ability to "age" (i.e., dealkylation of the dialkoxymethylphosphinyl enzyme). This dealkylation (hydrolysis) can produce a permanently inhibited, charged enzyme molecule thought to be the initiator of OPIDN (Johnson, 1975). Phosphinyl and sulfonyl compounds do not hydrolyze, whereas

carbamates undergo hydrolysis to yield an intermediate which decomposes to CO₂ and free enzyme. Conversely, phosphorus esters (R-O-P) or amide (R-N-P) structure types can hydrolyze leaving an ionized acidic group attached to the phosphorus atom.

Another confounding variable comes to light when the delayed neuropathic potential of OP compounds is considered. That consideration is the degree of AChE inhibition or acute OP poisoning potential of the compound. The greater that potential, the less the chance the animal will survive the acute cholinergic crisis-stage; thus, consideration of delayed neuropathic potential is a moot point. Again, there are studies on the structure-activity relationships regarding AChE inhibition (Eto, 1974) and also regarding the ratio of *in vitro* AChE/NTE inhibition (Lotti and Johnson, 1978). *In vivo* animal tests of suspect OP compounds usually do not protect or treat the acute poisoning stage of OP toxicity with reactivators and/or atropine administration. However, the case with human poisonings can be quite different. New and improved pharmaceuticals have been developed for the treatment and prevention of acute OP poisoning, so that humans exposed to a "lethal" dosage of OP pesticide could survive the acute stage only to be subject later to the delayed neuropathy. Therefore, toxicity testing of OP compounds in an unprotected avian species may sorely underestimate the delayed neuropathic potential of OP compounds in humans treated extensively during the acute cholinergic crisis.

Three questions might be considered regarding the biochemical activity of OP compounds:

- (1) Many of the organophosphates are manufactured for a specific biological activity: their ability to inhibit insect AChE. They are thought to induce OPIDN by activity at another, but perhaps similar, esterase. There is a great deal of structural and functional information on AChE and little of that type of information on the esterase involved with OPIDN. How would we go about devising a strategy to predict the differential inhibition of these two enzymes and therefore the differential activity of the organophosphates?
- (2) How can we devise a strategy for testing this model for the relationship between NTE and OPIDN?
- (3) Are there characteristics that will allow one to predict a chemical's potential to induce OPIDN?

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16. ABSTRACT On October 25 and 26, 1984, the U.S. EPA sponsored a workshop to consider the potential applications of the techniques of computational biological chemistry to problems in environmental health. Eleven extramural scientists from the various related disciplines and a similar number of Agency personnel participated. Briefing materials describing some specific Agency problems in this area were sent to the extramural scientists before the meeting. The workshop's goal was the development of a research strategy for these potential applications. At this workshop, four classes of problems were identified and the recommendations of the panel for each class were drafted separately. The four classes were as follows: 1) methodology for handling diverse structures, 2) methodology for handling data sets with substantial structural similarities, 3) theoretical structure-activity studies using causally related molecular parameters, and 4) application possibilities for artificial intelligence decision aids in the existing Premanufacture Notification (PMN) review process. The final document was a product of the entire panel. All members agreed on the need for experimental toxicity data sets designed for structure-activity relationship studies. The report contains the recommendations of the panel. The briefing materials are contained in the appendices.		
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